NIST IR 8154

Dietary Supplement Laboratory Quality Assurance Program: Exercise L Final Report

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ABSTRACT

The NIST Dietary Supplement Laboratory Quality Assurance Program (DSQAP) was established in collaboration with the National Institutes of Health (NIH) Office of Dietary Supplements (ODS) in 2007 to enable members of the dietary supplements community to improve the accuracy of measurements for demonstration of compliance with various regulations including the dietary supplement current good manufacturing practices (cGMPs). Exercise L of this program offered the opportunity for laboratories to assess their in-house measurements of nutritional elements (iodine), contaminants (lead and arsenic), water-soluble vitamins (biotin), fat-soluble vitamins (lutein and zeaxanthin), fatty acids (omega-3 and -6), and botanical marker compounds (chlorogenic acid, flavonoids, and naphthodianthrones) in foods and/or botanical dietary supplement ingredients and finished products.

INTRODUCTION

The dietary supplement industry in the US is booming, with two-thirds of adults considering themselves to be supplement users.[1] Consumption of dietary supplements, which includes vitamin and mineral supplements, represents an annual US expenditure of more than $40 billion. These figures represent an increasing American and worldwide trend, and as a result, it is critically important that both the quality and safety of these products are verified and maintained.

The Dietary Supplement Health and Education Act of 1994 (DSHEA) amended the Federal Food, Drug, and Cosmetic Act to create the regulatory category called dietary supplements. The DSHEA also gave the FDA authority to write current Good Manufacturing Practices (cGMPs) that require manufacturers to evaluate the identity, purity, and composition of their ingredients and finished products. In addition, the DSHEA authorized the establishment of the Office of Dietary Supplements at the National Institutes of Health (NIH ODS). To enable members of the dietary supplements community to improve the accuracy of the measurements required for compliance with these and other regulations, NIST established the Dietary Supplement Laboratory Quality Assurance Program (DSQAP) in collaboration with the NIH ODS in 2007.

The program offers the opportunity for laboratories to assess their in-house measurements of active or marker compounds, nutritional elements, contaminants (toxic elements, pesticides, mycotoxins), and fat- and water-soluble vitamins in foods as well as botanical dietary supplement ingredients and finished products. Reports and certificates of participation are provided and can be used to demonstrate compliance with the cGMPs. In addition, NIST and the DSQAP assist the ODS Analytical Methods and Reference Materials program (AMRM) at the NIH in supporting the development and dissemination of analytical tools and reference materials. In the future, results from DSQAP exercises could be used by ODS to identify problematic matrices and analytes for which an AOAC INTERNATIONAL Official Method of Analysis would benefit the dietary supplement community.

NIST has experience in the administration of quality assurance programs, but the DSQAP takes a unique approach. In other NIST quality assurance programs, a set of analytes is measured

[1] Walsh, T. (2012) *Supplement Usage, Consumer Confidence Remain Steady According to New Annual Survey from CRN*. Council for Responsible Nutrition, Washington, DC.

repeatedly over time in the same or similar matrices to demonstrate and improve laboratory performance. In contrast, the wide range of matrices and analytes under the "dietary supplement" umbrella means that not every laboratory is interested in every sample or analyte. The constantly changing dietary supplement market, and the enormous diversity of finished products, makes repeated determination of a few target compounds in a single matrix of little use to participants. Instead, participating laboratories are interested in testing in-house methods on a wide variety of challenging, real-world matrices to demonstrate that their performance is comparable to that of the community and that their methods provide accurate results. In an area where there are few standard methods, the DSQAP offers a unique tool for assessment of the quality of measurements, provides feedback about performance, and can assist participants in improving laboratory operations.

This report summarizes the results from the eleventh exercise of the DSQAP, Exercise L. Eighty-two laboratories responded to the call for participants distributed in October 2015. Samples were shipped to participants in January 2016, and results were returned to NIST by March 2016. This report contains the final data and information that was disseminated to the participants in October 2016.

OVERVIEW OF DATA TREATMENT AND REPRESENTATION

Individualized data tables and certificates are provided to the participants that have submitted data in each study, in addition to this report. Examples of the data tables using NIST data are also included in each section of this report. Community tables and graphs are provided using randomized laboratory codes, with identities known only to NIST and individual laboratories. The statistical approaches are outlined below for each type of data representation.

Statistics

Data tables and graphs throughout this report contain information about the performance of each laboratory relative to that of the other participants in this study and relative to a target around the expected result, if available. All calculations are performed in PROLab Plus (QuoData GmbH, Dresden, Germany).[2] The consensus mean and standard deviation are calculated according to the robust algorithm outlined in ISO 13528:2015(E), Annex C.[3] The algorithm is summarized here in simplified form.

Initial values of the consensus mean, x^*, and consensus standard deviation, s^*, are estimated as

$$x^* = \text{median of } x_i \qquad (i = 1, 2,\ldots,n)$$
$$s^* = 1.483 \times \text{median of } |x_i - x^*| \qquad (i = 1, 2,\ldots,n).$$

These initial values for x^* and s^* are updated by first calculating the expanded standard deviation, δ, as

$$\delta = 1.5 \times s^*.$$

[2] Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

[3] ISO 13528:2015(E), *Statistical methods for use in proficiency testing by interlaboratory comparisons*, pp. 53-54.

Then each x_i is compared to the expanded range and adjusted to x_i* as described below to reduce the effect of outliers.

If $x_i < x^* - \delta$, then $x_i^* = x^* - \delta$.
If $x_i > x^* + \delta$, then $x_i^* = x^* + \delta$.
Otherwise, $x_i^* = x_i$.

New values of x^*, s^*, and δ are calculated iteratively until the process converges. Convergence is taken as no change from one iteration to the next in the third significant figure of s^* and in the equivalent digit in x^*:

$$x^* = \frac{\sum_{i=1}^{n} x_i^*}{n}$$

$$s^* = 1.134 \times \sqrt{\frac{\sum_{i=1}^{n} (x_i^* - x^*)}{n-1}}.$$

Individualized Data Table

The data in this table is individualized to each participating laboratory and is provided to allow participants to directly compare their data to the summary statistics (consensus or community data as well as NIST certified, reference, or estimated values). The upper left of the data table includes the randomized laboratory code. Tables included in this report are generated using NIST data to protect the identity and performance of participants.

Section 1 of the data table contains the laboratory results as reported, including the mean and standard deviation when multiple values were reported. A blank indicates that NIST does not have data on file for that laboratory for a particular analyte or matrix. An empty box for standard deviation indicates that only a single value was reported and therefore that value was not included in the calculation of the consensus data.[3]

Also in Section 1 are two Z-scores. The first Z-score, Z'_{comm}, is calculated with respect to the community consensus value, taking into consideration bias that may result from the uncertainty in the assigned consensus value, using x* and s*:

$$Z'_{comm} = \frac{x_i - x*}{\sqrt{2} s*}$$

The second Z-score, Z_{NIST}, is calculated with respect to the target value (NIST certified, reference, or estimated value), using x_{NIST} and U_{95} (the expanded uncertainty) or s_{NIST} (the standard deviation of NIST measurements):

$$Z_{NIST} = \frac{x_i - x_{NIST}}{U_{95}}$$

or

$$Z_{NIST} = \frac{x_i - x_{NIST}}{s_{NIST}}.$$

The significance of the Z-score is as follows:

- $|Z| < 2$ indicates that the laboratory result is considered to be within the community consensus range (for Z'_{comm}) or NIST target range (for Z_{NIST}).
- $2 < |Z| < 3$ indicates that the laboratory result is considered to be marginally different from the community consensus value (for Z'_{comm}) or NIST target value (for Z_{NIST}).
- $|Z| > 3$ indicates that the laboratory result is considered to be significantly different from the community consensus value (for Z'_{comm}) or NIST target value (for Z_{NIST}).

Section 2 of the data table contains the community results, including the number of laboratories reporting more than a single value for a given analyte[1], the mean value determined for each analyte, and a robust estimate of the standard deviation of the reported values.[4] Consensus means and standard deviations are calculated using the laboratory means; if a laboratory reported a single value, the reported value is not included.[3] Additional information on calculation of the consensus mean and standard deviation can be found in the previous section.

Section 3 of the data table contains the target values for each analyte. When possible, the target value is a certified or reference value determined at NIST. Certified values and the associated expanded uncertainty (U_{95}) have been determined with two independent analytical methods at NIST, by collaborating laboratories, or in some combination. Reference values are assigned using NIST values obtained from the average and standard deviation of measurements made using a single analytical method or by measurements obtained from collaborating laboratories. For both certified and reference values, at least six samples have been tested and duplicate preparations from the sample package have been included, allowing the uncertainty to encompass variability due to inhomogeneity within and between packages. For samples in which a NIST certified or reference value is not available, the analytes are measured at NIST using an appropriate method. The NIST-assessed value represents the mean of at least three replicates. For materials acquired from another proficiency testing program, the consensus value and uncertainty from the completed round is used as the target range.

Summary Data Table

This data table includes a summary of all reported data for a particular analyte in a particular study. Participants can compare the raw data for a single laboratory to data reported by the other participating laboratories or to the consensus data. A blank indicates that the laboratory signed up and received samples for that particular analyte and matrix, but NIST does not have data on file for that laboratory.

Graphs

Data Summary View (Method Comparison Data Summary View)

In this view, individual laboratory data are plotted with the individual laboratory standard deviation (error bars). Laboratories reporting values below the method quantitation limit are shown in this view as downward triangles beginning at the limit of quantitation (LOQ). Laboratories reporting values as "below LOQ" can still be successful in the study if the target value is also below the laboratory LOQ. The black solid line represents the consensus mean, and

[4] ISO 13528:2015(E), *Statistical methods for use in proficiency testing by interlaboratory comparisons*, Annex C.

the green shaded area represents the consensus variability. Where appropriate, two consensus means may be calculated for the same sample if bimodality is identified in the data. In this case, two consensus means and ranges will be displayed in the data summary view. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified, reference, or estimated value bounded by twice its uncertainty (U_{95}) or standard deviation. For the purpose of the DSQAP, a target range spanning twice the uncertainty in the NIST value is selected because participants are only asked to make a limited number of observations. The size of the y-axis on the data summary view graph represents the range of tolerance (values that result in an acceptable Z' score, $|Z'| \leq 2$). In this view, the relative locations of individual laboratory data and consensus zones with respect to the target zone can be compared easily. In most cases, the target zone and the consensus zone overlap, which is the expected result. The major program goals are to reduce the size of the consensus zone and center the consensus zone about the target value. Analysis of an appropriate reference material as part of a quality control scheme can help to identify sources of bias for laboratories reporting results that are significantly different from the target zone. In the case in which a method comparison is relevant, different colored data points may be used to indicate laboratories that used a specific approach to sample preparation, analysis, or quantitation.

Sample/Sample Comparison View

In this view, the individual laboratory results for one sample (NIST SRM with a certified or reference value) are compared to the results for another sample (another NIST SRM with a more challenging matrix, a commercial sample, etc.). The solid red box represents the target zone for the first sample (x-axis) and the second sample (y-axis). The dotted blue box represents the consensus zone for the first sample (x-axis) and the second sample (y-axis). The axes of this graph are centered about the consensus mean values for each sample or control, to a limit of twice the range of tolerance (values that result in an acceptable Z' score, $|Z'| \leq 2$). Depending on the variability in the data, the axes may be scaled proportionally to better display the individual data points for each laboratory. In some cases, when the consensus and target ranges have limited overlap, the solid red box may only appear partially on the graph. If the variability in the data is high (greater than 100 % relative standard deviation (RSD)), the dotted blue box may also only appear partially on the graph. This view emphasizes trends in the data that may indicate potential calibration issues or method biases. One program goal is to identify such calibration or method biases and assist participants in improving analytical measurement capabilities. In some cases, when two equally challenging materials are provided, the same view (sample/sample comparison) can be helpful in identifying commonalities or differences in the analysis of the two materials.

NUTRITIONAL ELEMENTS (IODINE) IN CAT FOOD AND MULTIVITAMIN TABLETS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3290 Dry Cat Food and SRM 3280 Multivitamin/Multielement Tablets. Participants were asked to use in-house analytical methods to determine the mass fraction of iodine in each of the matrices and report values on an as-received basis.

Sample Information

Cat Food. Participants were provided with one packet containing approximately 10 g of dry cat food. The cat food was blended, aliquotted, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single packet provided. Approximate analyte levels were not reported to participants prior to the study. The reference value for iodine in SRM 3290 Dry Cat Food was determined at NIST using inductively coupled plasma mass spectrometry (ICP-MS) and instrumental neutron activation analysis (INAA). The reference values and uncertainties for iodine are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (4.36 %).

	Reference Mass Fraction in SRM 3290 (mg/kg)	
Analyte	(dry-mass basis)	(as-received basis)
Iodine (I)	3.38 ± 0.54	3.23 ± 0.52

Multivitamin. Participants were provided with one bottle containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind all tablets together and mix the resulting powder thoroughly, and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. The certified value for iodine in SRM 3280 Multivitamin/ Multielement Tablets was determined at NIST using inductively coupled plasma mass spectrometry (ICP-MS) and instrumental neutron activation analysis (INAA). The certified values and uncertainties for iodine are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (1.37 %).

	Certified Mass Fraction in SRM 3280 (mg/kg)	
Analyte	(dry-mass basis)	(as-received basis)
Iodine (I)	132.7 ± 6.6	130.9 ± 6.5

Study Results

- Thirty-three laboratories enrolled in this exercise and received samples. Eleven laboratories reported results for the multivitamin sample (33 % participation). Twelve laboratories reported results for the cat food sample (36 % participation). Ten and 11 laboratories were used, respectively, for calculation purposes, see Statistics, page 3.
- The consensus means for iodine in both materials were within the target range with acceptable between-laboratory variability (15 % to 20 % RSD).
- A majority of the laboratories reported using hot block digestion (33 %), microwave digestion (25 %), or solvent extraction (25 %) for sample preparation. The remaining laboratories reported using base hydrolysis or dry ashing, although no values were reported by the laboratory that reported using dry ashing.
- A majority of the laboratories reported using ICP-MS (69 %) as their analytical method. The remaining laboratories reported using ion-selective electrode, ion chromatography with conductivity detection, liquid chromatography, and thiosulfate titration, although no values were reported by the laboratory that reported using thiosulfate titration.

Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- The iodine study had the lowest enrollment (40 %) and participation rate (33 % to 36 %) of a nutritional elements study in the last five years. The nutritional elements studies are normally some of the most popular with the highest number of participants.
  - Over the past five years, nutritional elements studies have had 47 % to 65 % of total laboratories enrolled, with 65 % to 83 % participation.
  - The low participation in this study could be the result of a lack of interest in iodine or the greater challenge posed by analysis of iodine compared to other nutritional elements.
- With a small number of laboratories reporting data, identification of strong trends in the data based on the information reported by participants is difficult. The data suggest that ICP-MS and digestion sample preparations, acid or base, were slightly more successful than chromatography methods and solvent extractions.
- Some suggestions regarding iodine sample preparation are provided below.
  - Iodine is a volatile element and can form hydrogen iodide (HI) during acid digestion; care must be taken to retain iodine during sample preparation.
  - Iodine is also light sensitive and at some stages of sample preparation solutions may need to be kept in amber or covered samples vessels.
  - When using ICP-MS, an acidic sample solution can result in sample carryover. Using a basic solution or a surfactant such as Triton X-100 will improve washout of iodine. Some protocols use an alkaline digestion with tetramethylammonium hydroxide (TMAH), but extreme caution must be taken when using TMAH, which is a very strong base with high toxicity. A safer alternative may be to use an acid digestion then solutions can be neutralized with a base such as ammonium hydroxide.
  - During sample preparation, iodine can adhere to TFM vessels, so PFA vessels or quartz/glass vessels are recommended to eliminate erratic results.

Table 1. Individualized data summary table (NIST) for iodine in cat food and multivitamin tablets.

National Institute of Standards & Technology

Exercise L - October 2015 - Iodine

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Iodine	Multivitamin	mg/kg	131	13		0.0	10	131	20	131	13
Iodine	Cat Food	mg/kg	3.2	1.0		0.0	11	3.5	0.7	3.2	1.0

x_i Mean of reported values

s_i Standard deviation of reported values

Z'_{comm} Z'-score with respect to community consensus

Z_{NIST} Z-score with respect to NIST value

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 2. Data summary table for iodine in multivitamin tablets and cat food.

		Iodine									
		SRM 3280 MultivitaminTablets (mg/kg)					SRM 3290 Dry Cat Food (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				131	13				3.23	1.03
	L101										
	L102										
	L103	117	93		105	17	3.03	3.34		3.19	0.22
	L104										
	L105										
	L107	129	139	140	136	6	3.66	3.80	4.69	4.05	0.56
	L108										
	L110										
	L112										
	L115	126	123	123	124	2	3.52	3.61	3.45	3.53	0.08
	L117										
	L118	142	141	140	141	1	3.46	3.49	3.29	3.41	0.11
	L123	122	129	143	131	11	3.39	2.64	2.68	2.90	0.42
	L124										
	L126										
	L129						4.25	3.74	4.13	4.04	0.27
	L130										
	L136										
	L137	202	204	207	205	2	28.72	32.15	30.65	30.51	1.72
	L139	101	112	97	103	8	3.06	2.58	2.81	2.82	0.24
	L140										
	L141										
	L148										
	L151										
	L152	140			140		3.90			3.90	
	L155										
	L157										
	L159										
	L160	138	161	145	148	12	3.30	3.50	3.50	3.43	0.12
	L165										
	L170										
	L171										
	L172										
	L176										
	L177										
	L179	133	132	128	131	3	0.72	0.61	0.76	0.70	0.08
	L182	121	125	125	124	2	3.88	3.98	4.01	3.96	0.07
Community Results		Consensus Mean			131		Consensus Mean			3.52	
		Consensus Standard Deviation			20		Consensus Standard Deviation			0.70	
		Maximum			205		Maximum			30.51	
		Minimum			103		Minimum			0.70	
		N			10		N			11	

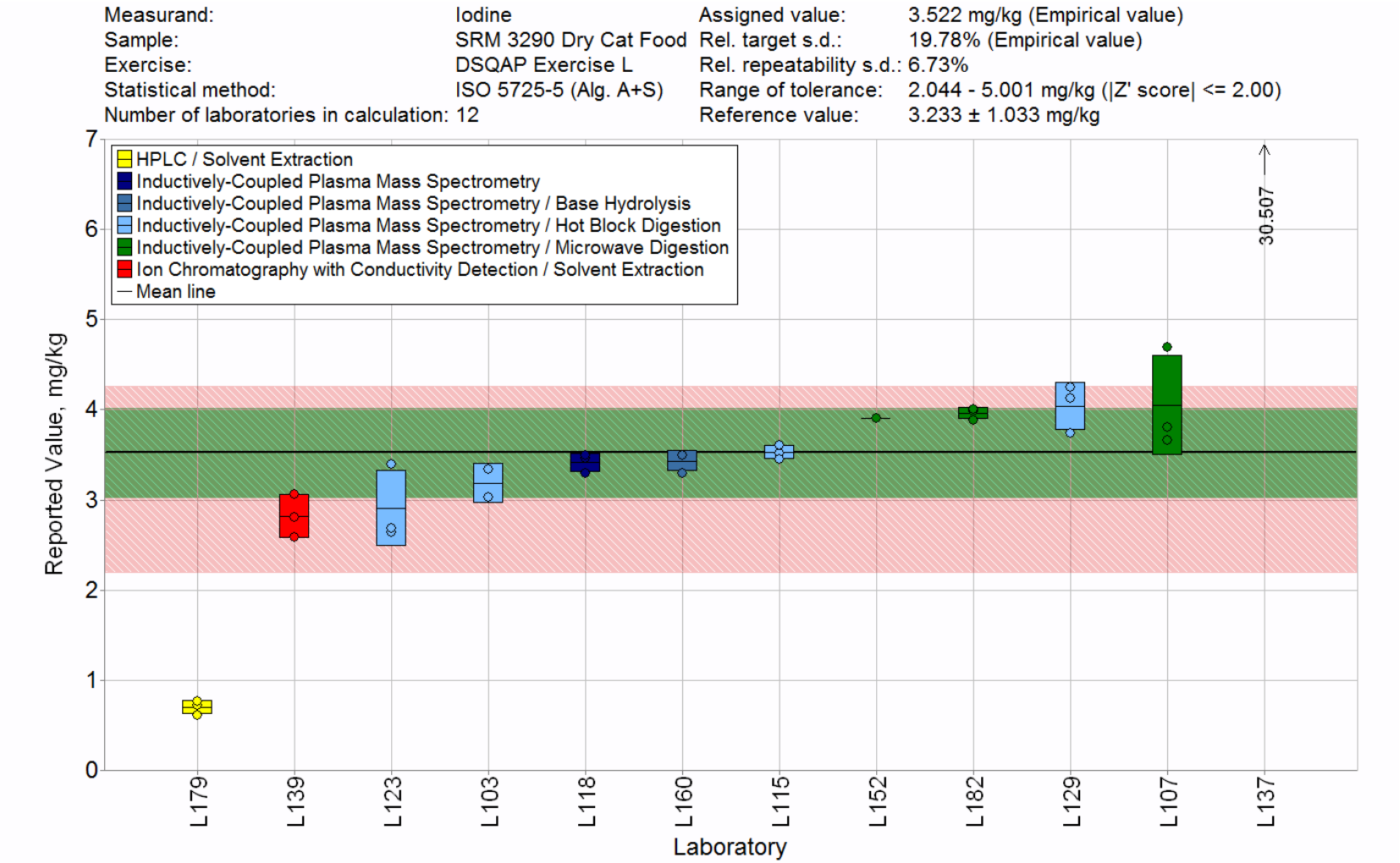

Figure 1. Iodine in SRM 3290 Dry Cat Food (data summary view – digestion and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation (digestion) procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

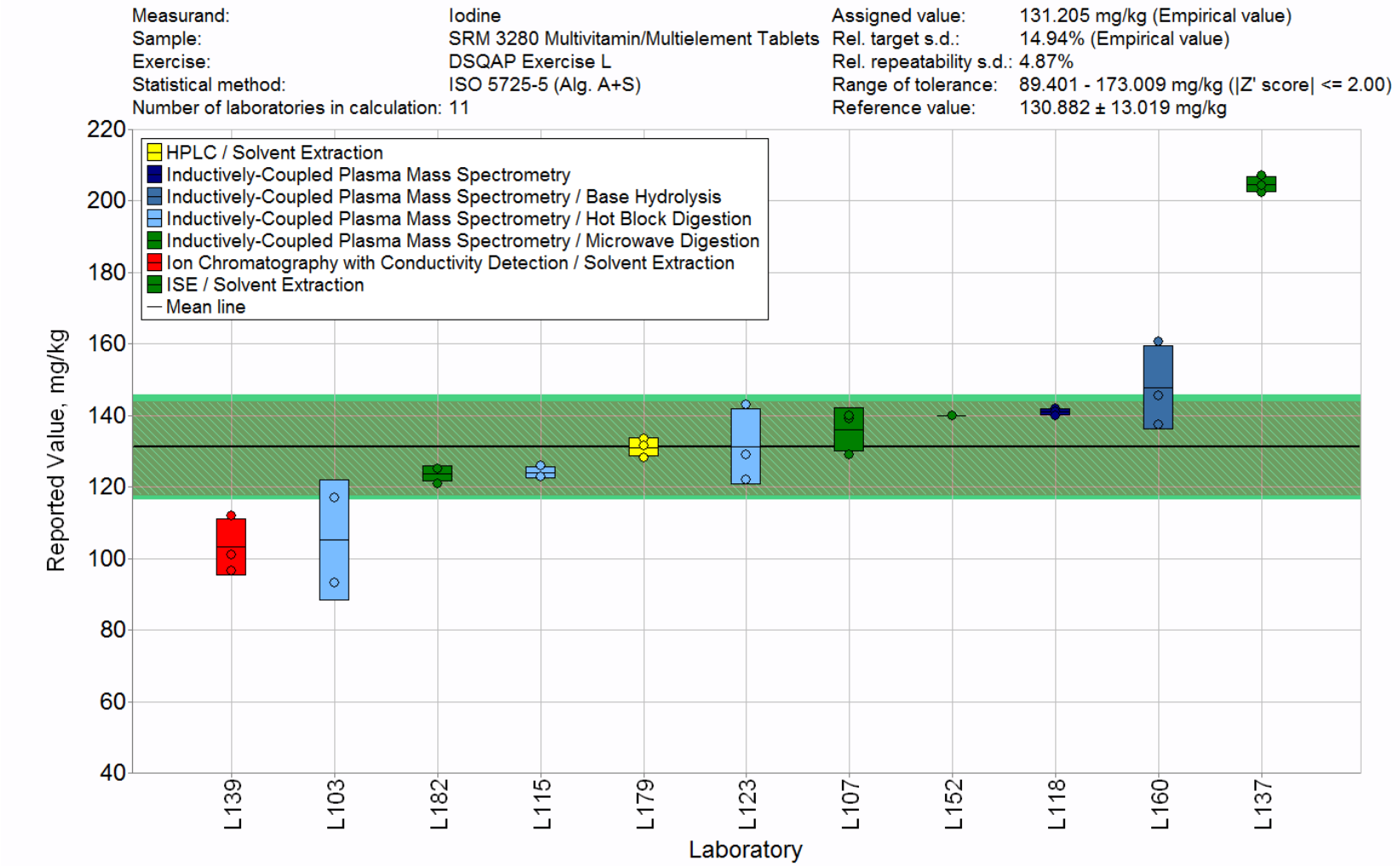

Figure 2. Iodine in SRM 3280 Multivitamin/Multielement Tablets (data summary view – digestion and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation (digestion) procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

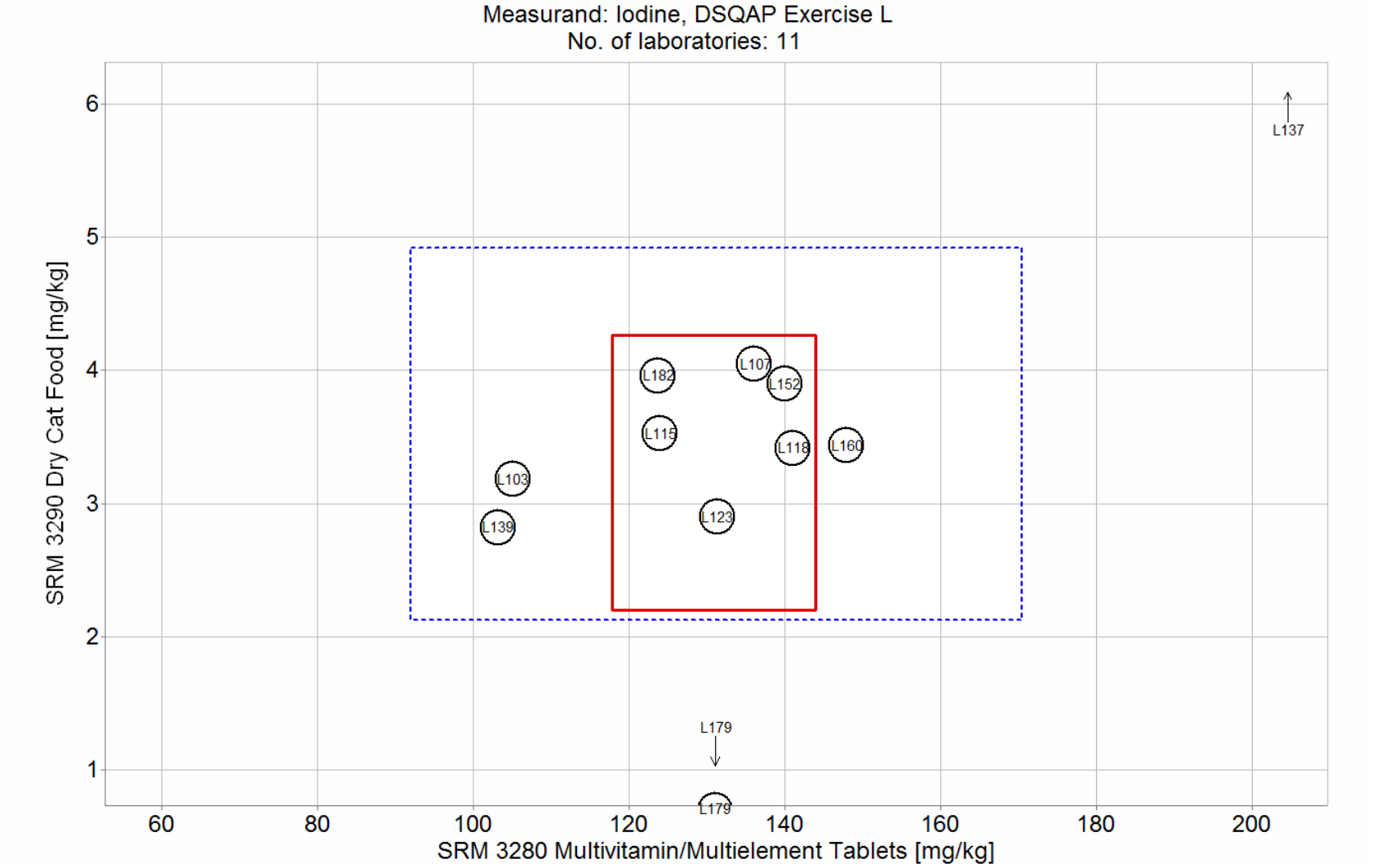

Figure 3. Iodine in SRM 3290 Dry Cat Food and SRM 3280 Multivitamin/Multielement Tablets (sample/sample comparison view). In this view, the individual laboratory results for one sample (multivitamin) are compared to the results for a second sample (cat food). The solid red box represents the target zone for the two samples, multivitamin (x-axis) and cat food (y-axis). The dotted blue box represents the consensus zone for multivitamin (x-axis) and cat food (y-axis).

TOXIC ELEMENTS (As AND Pb) IN ST. JOHN'S WORT DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract. Participants were asked to use in-house analytical methods to determine the mass fractions of total arsenic (As) and lead (Pb) in each of the matrices and report values on an as-received basis.

Sample Information

St. John's Wort Aerial Parts. Participants were provided with three packets containing approximately 3.3 g of dried St. John's Wort aerial parts. The dried leaves were ground, homogenized, and packaged inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and use a sample size of at least 1.0 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. The target value for arsenic in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts was determined at NIST using inductively coupled plasma mass spectrometry (ICP-MS) and instrumental neutron activation analysis (INAA). The target value for lead in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts was determined at NIST using isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS). The NIST-determined values and uncertainties for As and Pb are provided in the table below, on an as-received basis.

Analyte	NIST-Determined Mass Fraction in SRM 3262 (ng/g) (as-received basis)
Arsenic (As)	145 ± 13
Lead (Pb)	933 ± 137

St. John's Wort Methanol Extract. Participants were provided with three packets containing approximately 1.6 g of St. John's Wort methanol extract. The extract was ground, homogenized, and packaged inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and use a sample size of at least 0.6 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. The target value for arsenic in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract was determined at NIST using inductively coupled plasma mass spectrometry (ICP-MS). The certified value for lead in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract was determined at NIST using isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS). The NIST-determined value and uncertainty for As are provided in the table below, on an as-received basis. The certified values and uncertainties for Pb are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (0.92 %).

Analyte	NIST-Determined Mass Fraction in SRM 3264 (ng/g) (as-received basis)
Arsenic (As)	50 ± 18

Analyte	Certified Mass Fraction in SRM 3264 (ng/g) (dry-mass basis)	(as-received basis)
Lead (Pb)	30.3 ± 1.8	30.0 ± 1.8

Study Results

- Fifty-nine laboratories enrolled in this exercise and received samples.
  - Forty laboratories reported results for arsenic in St. John's wort aerial parts (68 % participation). Forty-two laboratories reported results for lead in St. John's wort aerial parts (71 % participation). Thirty-seven and 42 laboratories were used, respectively, for calculation purposes, see Statistics, page 3.
  - Thirty-seven laboratories reported results for arsenic in St. John's wort methanol extract (63 % participation). Thirty-eight laboratories reported results for lead in St. John's wort methanol extract (64 % participation). Thirty-four laboratories were used in both studies for calculation purposes, see Statistics, page 3.
- The consensus means for arsenic in the St. John's wort aerial parts and methanol extract were within the target ranges with high between-laboratory variability (23 % and 30 % RSD, respectively).
- The consensus mean for lead in the St. John's wort aerial parts was within the target range with acceptable between-laboratory variability (13 % RSD). The consensus mean for lead in the St. John's wort methanol extract was slightly above the target range with high between-laboratory variability (28 % RSD).
- For arsenic, a majority of the laboratories reported using microwave digestion (81 %) for sample preparation. Hot block digestion (14 %) and open beaker digestion (5 %) were also reported as methods of sample preparation.
- For lead, a majority of the laboratories also reported using microwave digestion (79 %) for sample preparation. Hot block digestion (14 %) and open beaker digestion (7 %) were also reported as methods of sample preparation.
- For arsenic, most laboratories reported using ICP-MS as their analytical method for analysis (90 %). Laboratories also reported using AAS (5 %) and ICP-OES (5 %).
- For lead, most laboratories also reported using ICP-MS as their analytical method for analysis (88 %). Laboratories also reported using AAS (9 %) and ICP-OES (2 %).

Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- Loss of volatile species of As is a concern and care must be taken not to lose As during sample preparation.
  - With a vigorous microwave digestion (81 % reported using microwave sample preparation) the high temperatures should convert all volatile organoarsenic species to arsenate As(V). At this point any subsequent heating will not result in loss of arsenic.

- Some laboratories performed well on the plant material but reported values with a high bias for the lower-level extract material.
  - More accurate measurements can be achieved using a calibration curve which closely surrounds the low concentrations found in these sample solutions.
  - The concentrations of the sample solutions must lie within the linear section of the calibration curve to prevent erroneous results. For a result outside the calibration range, multiplication by a dilution factor will only magnify the error.
- Some laboratories reported values within the target range for As in the extract material but reported low values in the plant material.
  - Ensure that samples are completely digested; higher temperatures or a stronger acid such as HF may be needed for plant materials.
  - Ensure that As is not lost during sample preparation either during inadvertent venting of vessels or when open beaker digestion is used.
- Lead is easily digested and volatile loss of Pb is not a concern. However, digestion with HCl may form a highly insoluble $PbCl_2$ precipitate. Digestion with HNO_3 is recommended for Pb analysis, or dry ashing with a small volume of acid.
- ICP-MS or AAS are recommend for analysis of low levels of As and Pb. Sensitivity of As and Pb is poor when using ICP-OES, possible pre-concentration of sample solutions to overcome poor sensitivity may be of use but extra care should be taken to overcome any additional contamination issues.
- An appropriate number of procedural blanks are important, and can be critical when sample concentrations are near the detection limit.

Table 3. Individualized data summary table (NIST) for arsenic and lead in St. John's wort (SJW) dietary supplements.

National Institute of Standards & Technology

Exercise L - October 2015 - Toxic Elements

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Lead (Pb)	SJW Aerial Parts	ng/g	933	273		0.00	42	825	106	933	273
Lead (Pb)	SJW Extract	ng/g	30.0	3.6		0.00	34	34.0	9.0	30.0	3.6
Arsenic (As)	SJW Aerial Parts	ng/g	145	26		0.00	37	129	30	145	26
Arsenic (As)	SJW Extract	ng/g	49.6	36.0		0.00	34	41.9	12.4	49.6	36.0

x_i Mean of reported values

s_i Standard deviation of reported values

Z'_{comm} Z'-score with respect to community consensus

Z_{NIST} Z-score with respect to NIST value

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 4. Data summary table for arsenic in St. John's wort dietary supplements.

		Total Arsenic									
		SRM 3262 St. John's Wort Aerial Parts (ng/g)					SRM 3264 St. John's Wort Extract (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				145	26				49.6	36.0
	L102	< 500.0	< 500.0	< 500.0			< 500.0	< 500.0	< 500.0		
	L103	123	154		139	22	< 60.0	< 60.0			
	L104										
	L105										
	L106	125	121	121	122	2	34.1	38.9	35.2	36.1	2.5
	L107	178	182	187	182	5	40.9	42.2	40.9	41.3	0.8
	L108	100	101	101	101	1	27.0	28.0	28.0	27.7	0.6
	L109	113	108	107	110	3	31.8	31.6	32.5	32.0	0.5
	L110	144	144	148	145	2	78.8	75.8	76.2	76.9	1.6
	L111	123	112	105	113	9	55.0	45.0	54.0	51.3	5.5
	L112										
	L115	119	129	126	125	5	32.0	31.0	31.0	31.3	0.6
	L116	150	152	146	149	3	36.0	38.0	39.0	37.7	1.5
	L117										
	L118	160	140	120	140	20	50.0	40.0	40.0	43.3	5.8
	L120	108	109	108	108	1	32.6	36.4	33.4	34.1	2.0
	L122										
	L123	168	155	147	157	11	39.9	42.2	48.5	43.5	4.5
	L124										
	L125	164	156		160	6	40.0	38.4	36.9	38.4	1.6
	L126	103	101	97	100	3	32.7	32.8	34.9	33.5	1.2
	L127	< 5.0	< 5.0	< 5.0			6.5	11.9	< 5.0	9.2	3.8
	L128	114	122	105	114	9	94.0	90.0	93.0	92.3	2.1
	L129	160	160	170	163	6					
	L130										
	L131										
	L132										
	L134										
	L136	102	104	98	101	3	23.5	22.5	22.9	23.0	0.5
	L137	98	100	99	99	1	43.0	45.0	42.7	43.6	1.3
	L138	173	181	186	180	7	37.4	50.9	49.1	45.8	7.3
	L139	80	80	70	77	6	37.3	30.6	40.5	36.1	5.1
	L140	114	112	122	116	5	37.0	38.4	36.1	37.2	1.2
	L141	119	114	121	118	4	38.2	35.6	37.4	37.1	1.3
	L142	87	77	91	85	7					
	L143										
	L144	91	99	81	90	9	58.0	37.0	40.0	45.0	11.4
	L145	142	143	147	144	3	43.7	44.4	41.5	43.2	1.5
	L147	134	139	172	148	21	37.0	35.5	219.8	97.4	106.0
	L148	141	148	147	145	4	62.3	70.5	67.3	66.7	4.1
	L151	108	105	112	108	3	36.3	32.7	41.3	36.8	4.3
	L154										
	L155	114	111	106	110	4	36.8	24.4	35.9	32.4	6.9
	L156										
	L157	108	112	114	111	3	32.9	37.0	34.3	34.7	2.1
	L158	138	141	141	140	2	49.0	57.0	48.0	51.3	4.9
	L159										
	L160	140	130	151	140	11	36.4	40.3	40.2	39.0	2.2
	L161										
	L162										
	L165	140	170	170	160	17	40.0	40.0	30.0	36.7	5.8
	L166										
	L167	149	141	151	147	5	95.5	89.0	101.0	95.2	6.0
	L169	162	157	157	159	3	69.8	71.3	67.1	69.4	2.1
	L170	107	105	98	103	5	45.0	49.0	49.0	47.7	2.3
	L171										
	L172										
	L173	215	220	220	218	3					
	L174										
	L175										
	L177										
	L179	< 330.0	< 330.0	< 330.0			< 520.0	< 520.0	< 520.0		
	L180										
	L181										
Community Results		Consensus Mean			129		Consensus Mean			41.9	
		Consensus Standard Deviation			30		Consensus Standard Deviation			12.4	
		Maximum			218		Maximum			97.4	
		Minimum			77		Minimum			9.2	
		N			37		N			34	

Table 5. Data summary table for lead in St. John's wort dietary supplements.

		Lead									
		SRM 3262 St. John's Wort Aerial Parts (ng/g)					SRM 3264 St. John's Wort Extract (ng/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				933	273				30.0	3.6
	L102	899	834	885	873	34	< 200.0	< 200.0	< 200.0		
	L103	800	811		806	8	< 60.0	< 60.0			
	L104										
	L105										
	L106	889	760	879	843	72	28.8	28.3	27.3	28.1	0.8
	L107	748	722	741	737	13	25.8	26.6	25.8	26.1	0.5
	L108	697	693	693	694	2	32.0	32.0	32.0	32.0	0.0
	L109	763	744	680	729	43	38.6	40.3	26.1	35.0	7.8
	L110	819	851	849	840	18	42.0	41.4	43.0	42.1	0.8
	L111	866	791	769	809	51	30.0	29.0	31.0	30.0	1.0
	L112										
	L115	796	767	831	798	32	28.0	27.0	29.0	28.0	1.0
	L116	862	884	887	878	14	32.0	35.0	32.0	33.0	1.7
	L117										
	L118	730	810	870	803	70	30.0	30.0	30.0	30.0	0.0
	L120	965	729	814	836	120	37.5	37.5	41.1	38.7	2.1
	L122										
	L123	756	725	849	777	65	24.5	24.0	22.8	23.8	0.9
	L124										
	L125	850	887		869	26	29.0	31.7	32.1	30.9	1.7
	L126	757	771	634	721	75	38.5	35.8	38.5	37.6	1.6
	L127	714	714	896	775	105	< 5.0	< 5.0	< 5.0		
	L128	997	946	999	981	30	35.0	32.0	33.0	33.3	1.5
	L129	980	990	930	967	32					
	L130										
	L131										
	L132										
	L134										
	L136	683	637	640	653	26	49.5	37.0	41.3	42.6	6.4
	L137	761	790	748	766	22	66.7	67.1	74.1	69.3	4.2
	L138	986	952	904	947	41	59.6	81.2	41.2	60.7	20.0
	L139	680	670	670	673	6	24.7	23.0	24.1	23.9	0.9
	L140	755	721	860	779	72	26.9	28.8	31.1	28.9	2.1
	L141	744	795	834	791	45	33.3	31.6	32.8	32.6	0.9
	L142	815	922	968	902	79			45.0	45.0	
	L143										
	L144	800	898	898	865	57	118.0	126.0	115.0	119.7	5.7
	L145										
	L147	807	868	852	842	32	25.6	24.2	26.5	25.4	1.2
	L148	740	842	671	751	86	28.2	25.7	24.9	26.3	1.7
	L151	698	715	745	719	24	44.2	50.5	43.8	46.2	3.8
	L154	1000	988	1008	999	10					
	L155	813	756	744	771	37	27.6	22.9	25.9	25.5	2.4
	L156										
	L157	689	779	845	771	78	27.2	29.1	37.0	31.1	5.2
	L158	796	981	1170	982	187	32.0	32.0	31.0	31.7	0.6
	L159										
	L160	756	823	909	829	77	28.9	28.5	28.3	28.6	0.3
	L161										
	L162										
	L165	860	920	830	870	46	30.0	40.0	40.0	36.7	5.8
	L166										
	L167	813	806	712	777	56	58.9	67.5	69.1	65.2	5.5
	L169	921	947	947	938	15	45.1	45.6	46.9	45.9	0.9
	L170	693	773	645	704	65	26.0	26.0	29.0	27.0	1.7
	L171										
	L172										
	L173	772	778	775	775	3	20.3	20.5	21.0	20.6	0.4
	L174										
	L175										
	L177										
	L179	940	810	860	870	66	25.0	29.0	28.0	27.3	2.1
	L180	1050	1000	1010	1020	26					
	L181	1050	1000	1010	1020	26					
Community Results		Consensus Mean			825		Consensus Mean			34.0	
		Consensus Standard Deviation			106		Consensus Standard Deviation			9.0	
		Maximum			1020		Maximum			119.7	
		Minimum			653		Minimum			20.6	
		N			42		N			34	

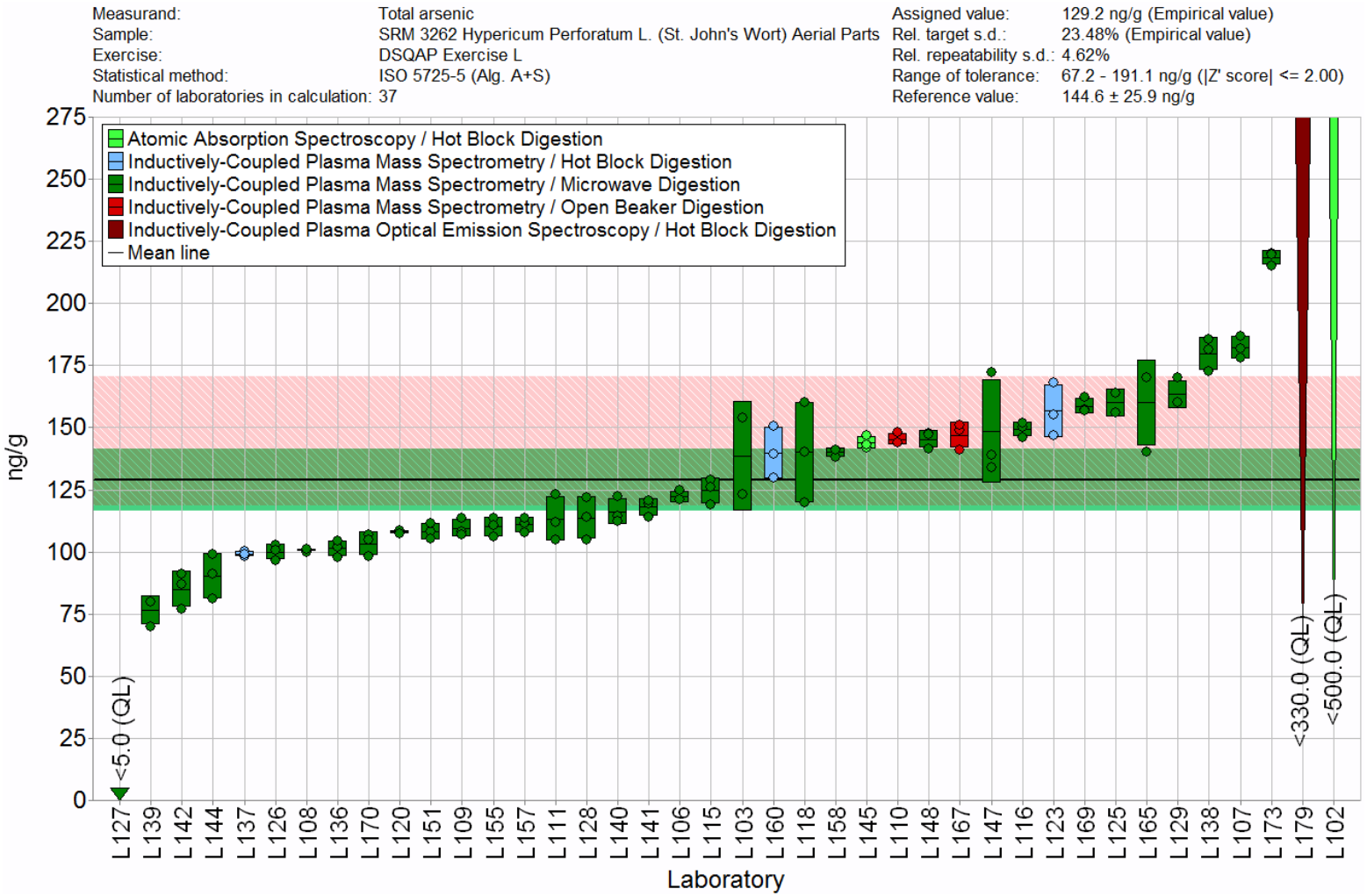

Figure 4. Arsenic in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – digestion and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation (digestion) procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

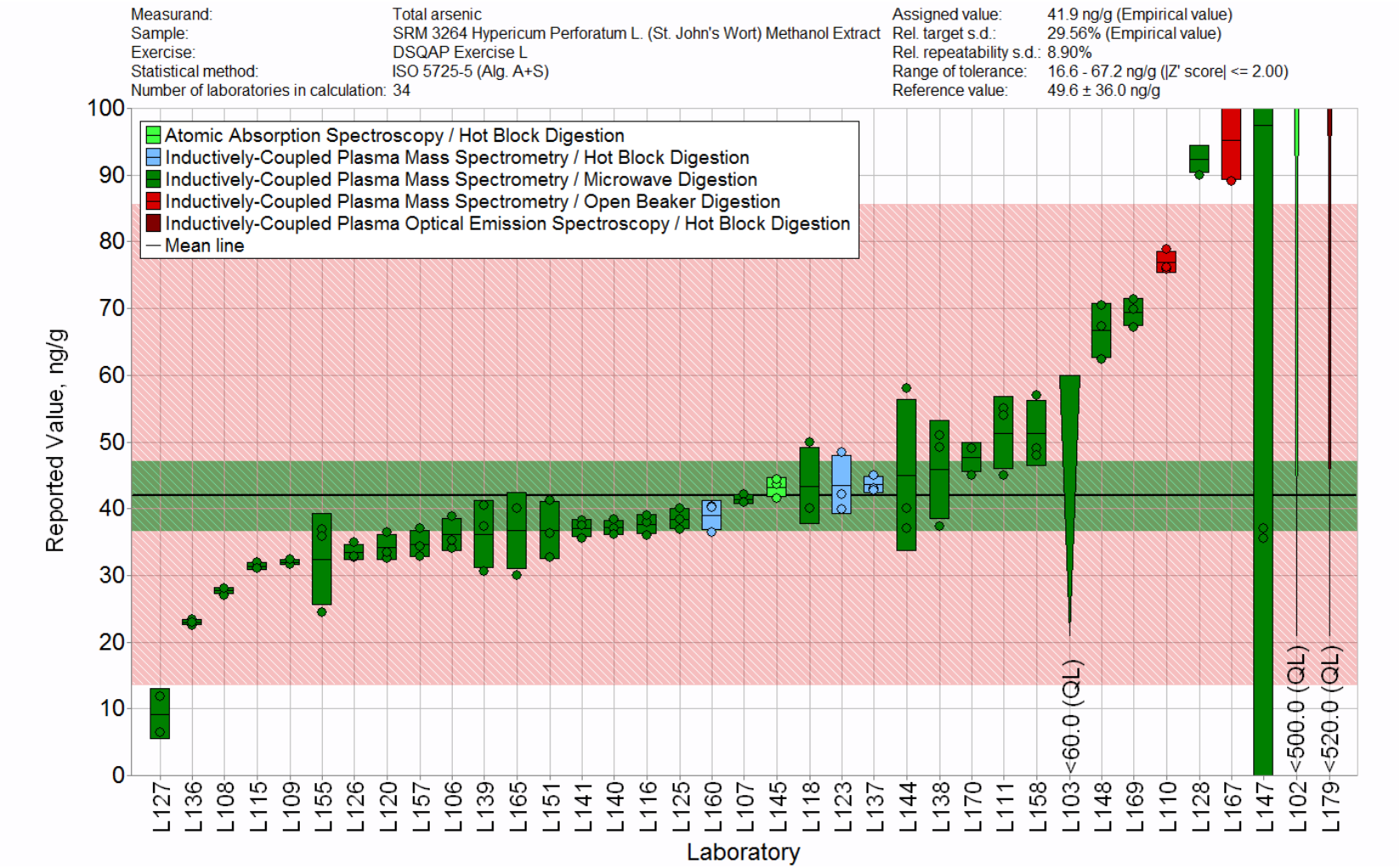

Figure 5. Arsenic in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – digestion and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation (digestion) procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

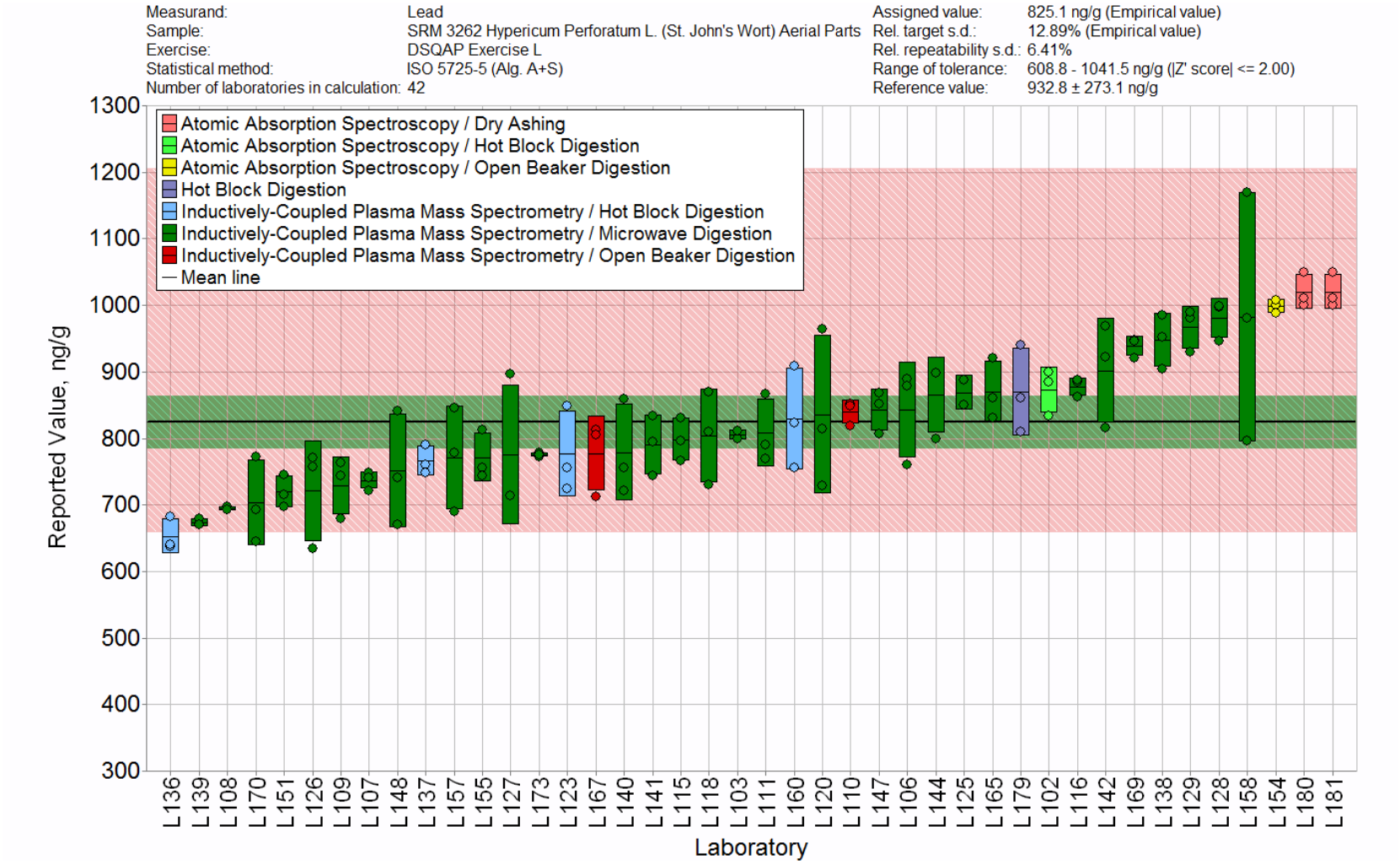

Figure 6. Lead in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – digestion and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation (digestion) procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

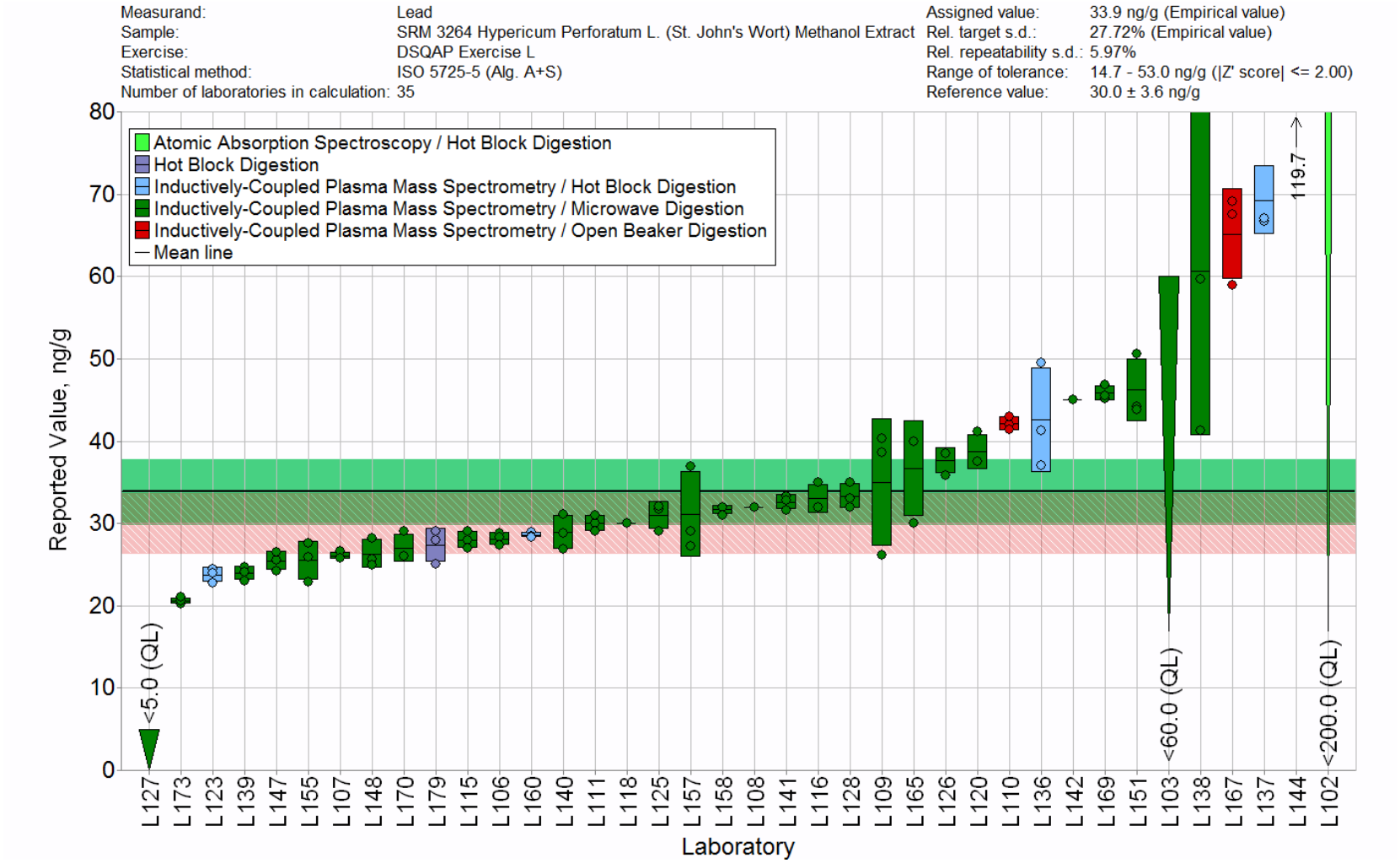

Figure 7. Lead in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – digestion and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation (digestion) procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

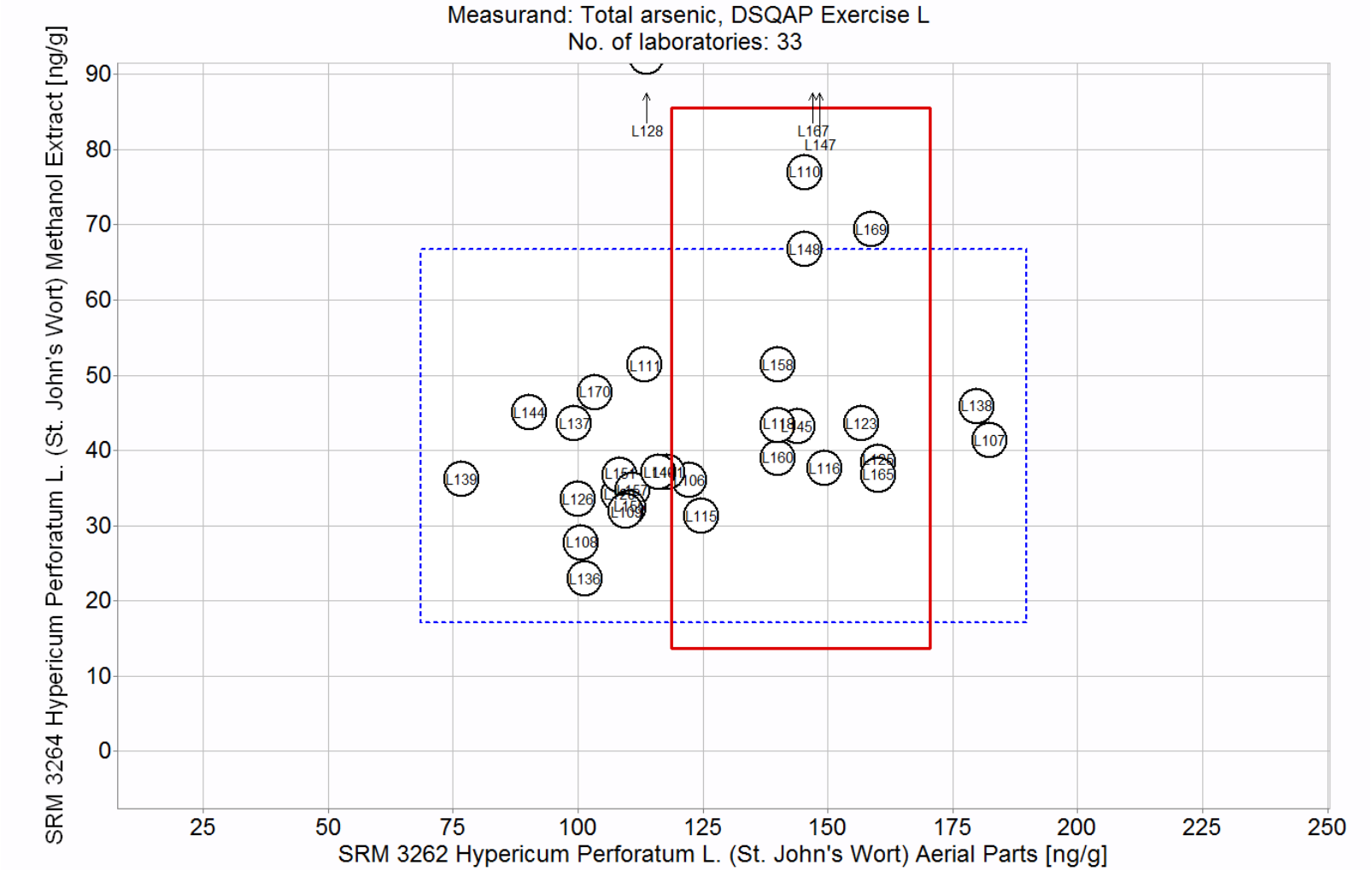

Figure 8. Arsenic in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The solid red box represents the target zone for the two samples, St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

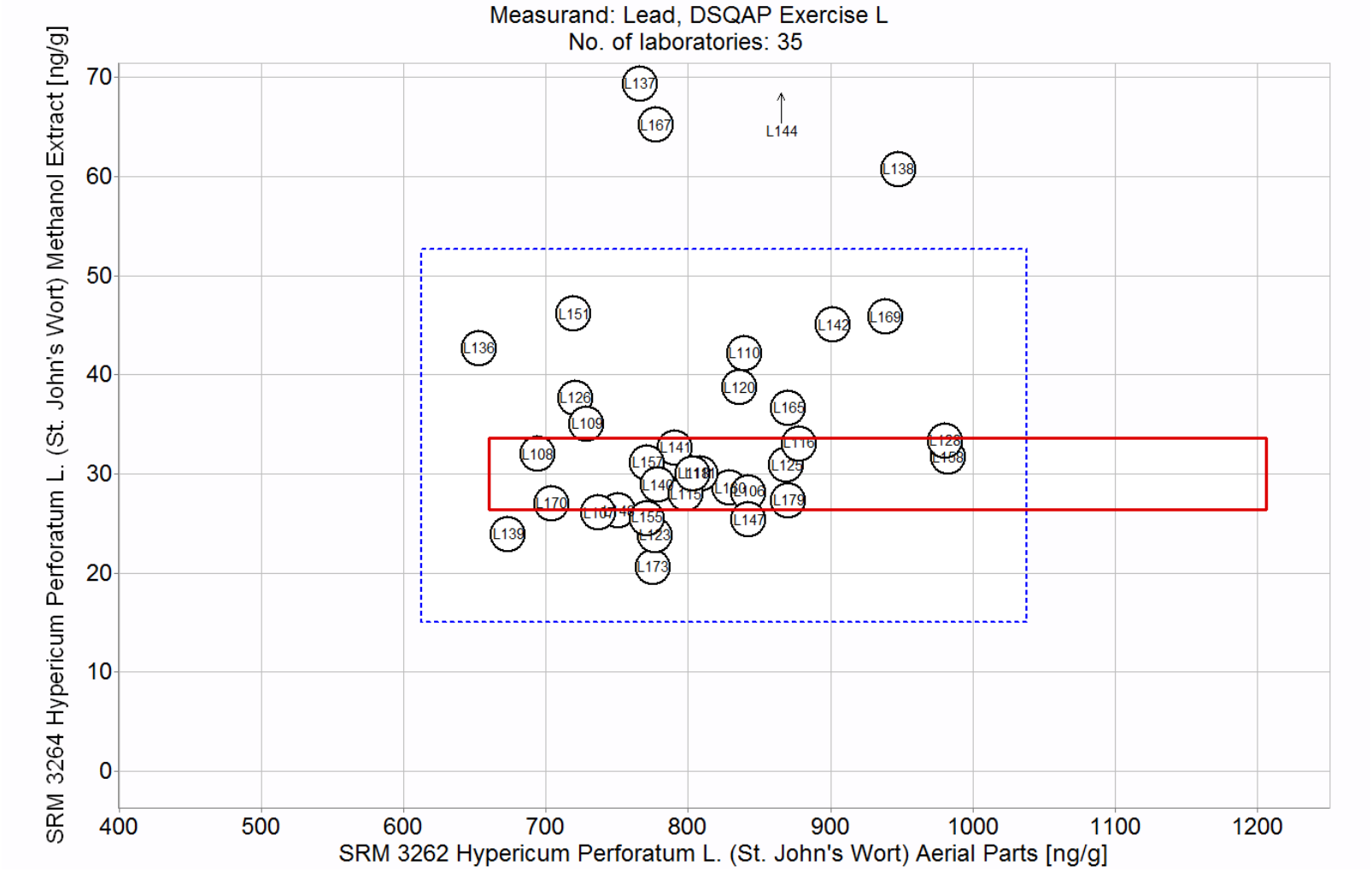

Figure 9. Lead in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The solid red box represents the target zone for the two samples, St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

WATER-SOLUBLE VITAMINS (BIOTIN) IN DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3290 Dry Cat Food and SRM 3280 Multivitamin/Multielement Tablets. Participants were asked to use in-house analytical methods to determine the mass fraction of biotin in each of the matrices and report values on an as-received basis.

Sample Information

Cat Food. Participants were provided with one packet containing approximately 10 g of dry cat food. The cat food was blended, aliquotted, and heat-sealed inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and to use a sample size of at least 0.5 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single packet provided. Approximate analyte levels were not reported to participants prior to the study. The certified value for biotin in SRM 3290 Dry Cat Food was determined at NIST using isotope dilution liquid chromatography mass spectrometry (ID-LC-MS), in combination with data from numerous collaborating laboratories. The certified values and uncertainties for biotin are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (4.36 %).

	Certified Mass Fraction in SRM 3290 (mg/kg)	
Analyte	(dry-mass basis)	(as-received basis)
Biotin	1.42 ± 0.23	1.36 ± 0.22

Multivitamin. Participants were provided with one bottle containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind all tablets together and mix the resulting powder thoroughly, and to use a sample size of at least 1.0 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to prepare three samples and report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. The certified value for biotin in SRM 3280 Multivitamin/ Multielement Tablets was determined at NIST using isotope dilution liquid chromatography mass spectrometry (ID-LC-MS), in combination with data from numerous collaborating laboratories. The certified values and uncertainties for biotin are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (1.37 %).

	Certified Mass Fraction in SRM 3280 (mg/kg)	
Analyte	(dry-mass basis)	(as-received basis)
Biotin	23.4 ± 3.2	23.1 ± 3.2

Study Results

- Forty laboratories enrolled in this exercise and received samples. Twenty-one laboratories reported results for SRM 3290 (53 % participation) and 23 laboratories reported results for

SRM 3280 (58 % participation). Nineteen and 23 laboratories were used, respectively, for calculation purposes, see Statistics, page 3.

- The consensus mean was within the target range for biotin in the multivitamin with acceptable between-laboratory variability (20 % RSD).
- The consensus mean was above the target range for biotin in the cat food with very high between-laboratory variability (61 % RSD).
- A majority of the laboratories reported using solvent extraction (78 %) as the sample preparation method. Laboratories also reported using dilution (13 %) and no sample preparation (9 %).
- A majority of the laboratories reported using liquid chromatography with mass spectrometry (48 %) as their instrumental method for analysis. Use of LC with absorbance detection (35 %), LC with tandem mass spectrometry (9%), HPLC (4 %), and microbiological assay (4 %) were also reported.

Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- Results for the multivitamin tablet were excellent. No methods presented as significantly better or worse than any other. No systematic biases were noted.
- For the cat food matrix, laboratories utilizing highly specific tandem mass spectrometry methods reported the most accurate results compared to the target value. The results from the single laboratory reporting use of microbiological assay were also consistent with the target value.
- Several laboratories reported values in the target range for the multivitamin tablet but high results for the cat food, indicating a potential challenge with the cat food matrix.
- Many of the laboratories reporting near the consensus mean, but higher than the target range, reported using LC-MS based methods. The high bias could be a result of a coelution or ion enhancement/suppression effects if an appropriate internal standard is not utilized.
- Extreme outliers in the measurement of biotin are likely a result of lack of specificity in the instrumental method.
    - All of the outlying laboratories reporting extremely high values used LC-absorbance methods.
    - Some laboratories using LC-absorbance may be experiencing a co-elution that would cause a high bias in the results. The problem can likely be corrected by alteration of the chromatographic conditions. The following recommendations can help identify and avoid potential coelutions.
        - A chromatographic method with alternate selectivity (different retention order) can be used as a check for each new sample type that is run. Ideally, the retention of coeluting compounds would also be affected and the results from the two chromatographic systems would be different. Two different responses would indicate a possible bias in one approach.
        - A different detector can be used in series with an absorbance detector (as confirmation), such as a fluorescence detector or mass spectrometer. If a coeluting compound is present, the response from these detectors would be different than the response from the absorbance detector. Two different responses would indicate a possible bias in one approach.

- Considerations of potential interferences can assist in troubleshooting. Understanding the matrix that is being tested and possible coeluting compounds can be evaluated before a sample is analyzed for additional confidence in the result.

Table 6. Individualized data summary table (NIST) for biotin in dietary supplements.

National Institute of Standards & Technology

Exercise L - October 2015 - Biotin

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Biotin	Multivitamin	mg/kg	23.1	6.3		0.00	23	23.9	4.9	23.1	6.3
Biotin	Cat Food	mg/kg	1.36	0.44		0.00	19	2.39	1.45	1.36	0.44

x_i Mean of reported values

s_i Standard deviation of reported values

Z'_{comm} Z'-score with respect to community consensus

Z_{NIST} Z-score with respect to NIST value

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 7. Data summary table for biotin in dietary supplements.

		Biotin									
		SRM 3280 MultivitaminTablets (mg/kg)					SRM 3290 Dry Cat Food (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				23.1	6.3				1.36	0.44
	L104										
	L105										
	L107	23.7	22.7	24.5	23.6	0.9	1.32	1.26	1.33	1.30	0.04
	L108										
	L110	24.0	22.6	22.7	23.1	0.8	1.27	1.18	1.26	1.24	0.05
	L111	20.6	20.6	20.8	20.7	0.1					
	L112										
	L116	21.6	22.1	21.4	21.7	0.4	1.50	1.70	1.70	1.63	0.12
	L117										
	L118	21.2	21.1	21.4	21.2	0.2	1.23	1.28	1.21	1.24	0.04
	L120	23.1	22.1	22.8	22.7	0.5	1.31	1.25	1.58	1.38	0.18
	L121						25.40	24.20	24.90	24.83	0.60
	L122										
	L123	30.3	33.1	34.3	32.6	2.1	3.66	4.00	4.03	3.90	0.21
	L124										
	L125	23.7	23.1		23.4	0.4					
	L126	23.1	23.4	23.8	23.4	0.4	3.88	3.62	3.93	3.81	0.17
	L127	71600.0	68667.0	68000.0	69422.3	1915.2	< 50.000	< 50.000	< 50.000		
	L128	40.9	41.8	41.5	41.4	0.5	112.00	112.00	111.00	111.67	0.58
	L130										
	L134										
	L137	116.9	120.8	134.8	124.1	9.4	7.22	7.58	6.85	7.22	0.37
	L138	22.0	22.0	22.0	22.0	0.0	1.60	1.60	1.30	1.50	0.17
	L139	23.6	23.1	23.1	23.3	0.3	1.10	1.12	1.07	1.10	0.03
	L140	23.2	23.1	23.2	23.2	0.1	1.83	1.74	1.66	1.74	0.09
	L141	24.7	24.6	25.5	24.9	0.5	2.08	2.38	2.12	2.19	0.16
	L142										
	L148										
	L151	22.6	22.7	23.1	22.8	0.3	2.91	2.98	2.92	2.93	0.04
	L153										
	L155	22.5	24.1	26.2	24.3	1.9	1.76	1.90	2.03	1.90	0.14
	L157	20.8	21.6	21.1	21.1	0.4	3.00	2.74	2.85	2.86	0.13
	L158	15.5	14.1	14.3	14.6	0.8	1.41	1.38	1.45	1.41	0.04
	L159										
	L160										
	L166										
	L168	20.2	20.8	21.4	20.8	0.6	1.26	1.39	1.40	1.35	0.08
	L170	30.6	29.9	29.1	29.9	0.8					
	L171										
	L172										
	L177										
	L178										
	L179	10.9	11.2	12.1	11.4	0.6	< 0.380	< 0.380	< 0.380		
Community Results		Consensus Mean			23.9		Consensus Mean			2.39	
		Consensus Standard Deviation			4.9		Consensus Standard Deviation			1.45	
		Maximum			69422.3		Maximum			111.67	
		Minimum			11.4		Minimum			1.10	
		N			23		N			19	

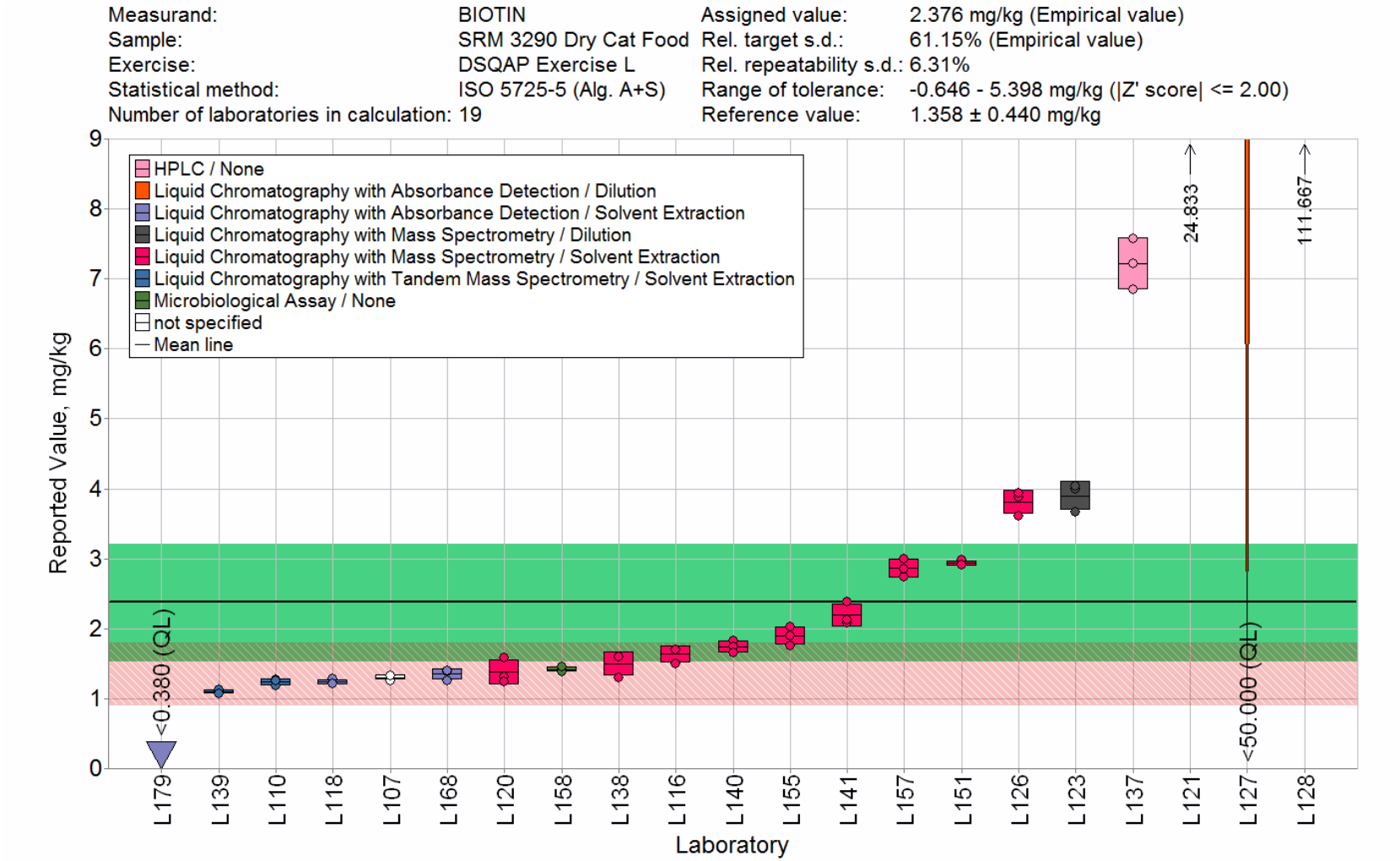

Figure 10. Biotin in SRM 3290 Dry Cat Food (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

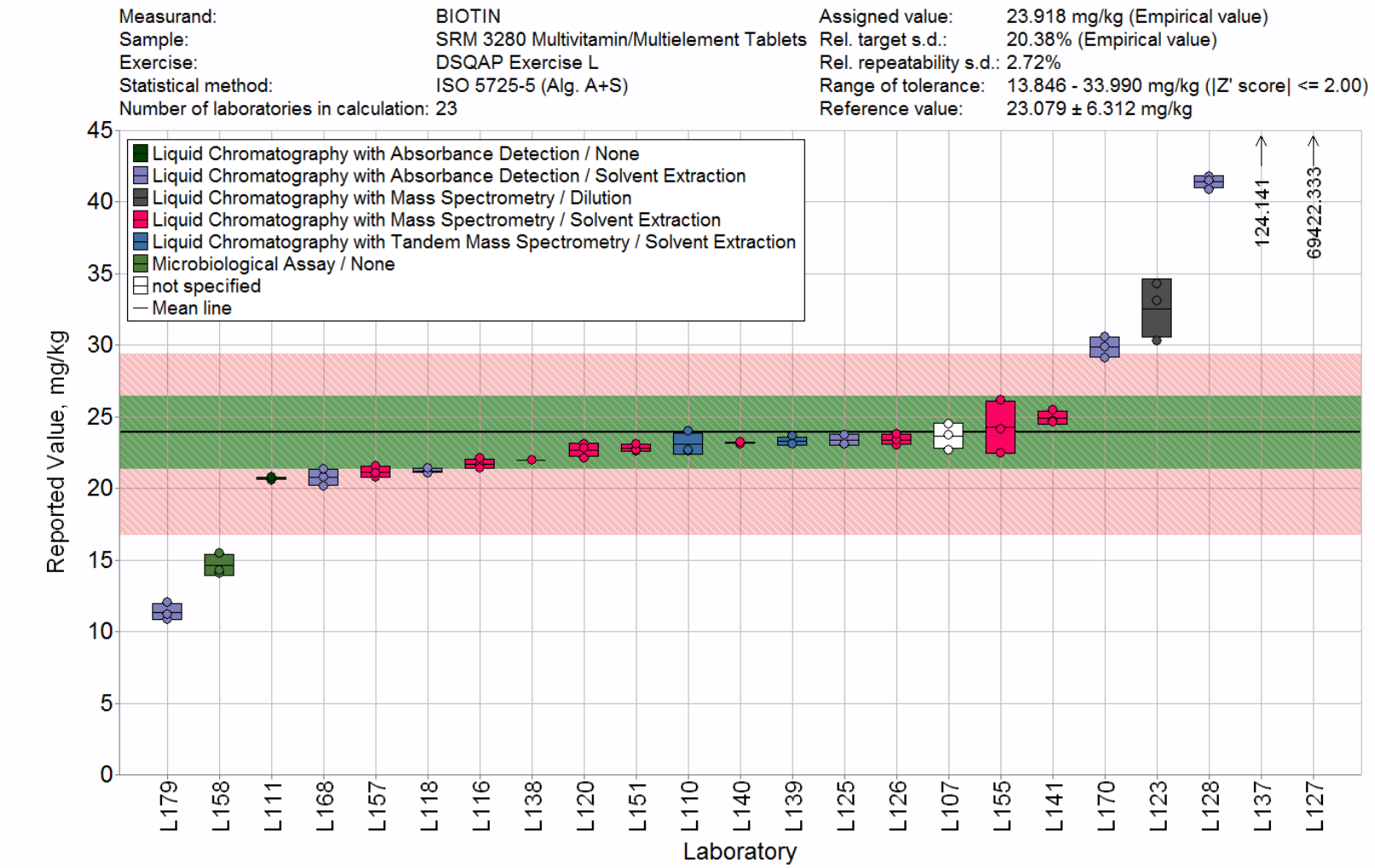

Figure 11. Biotin in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

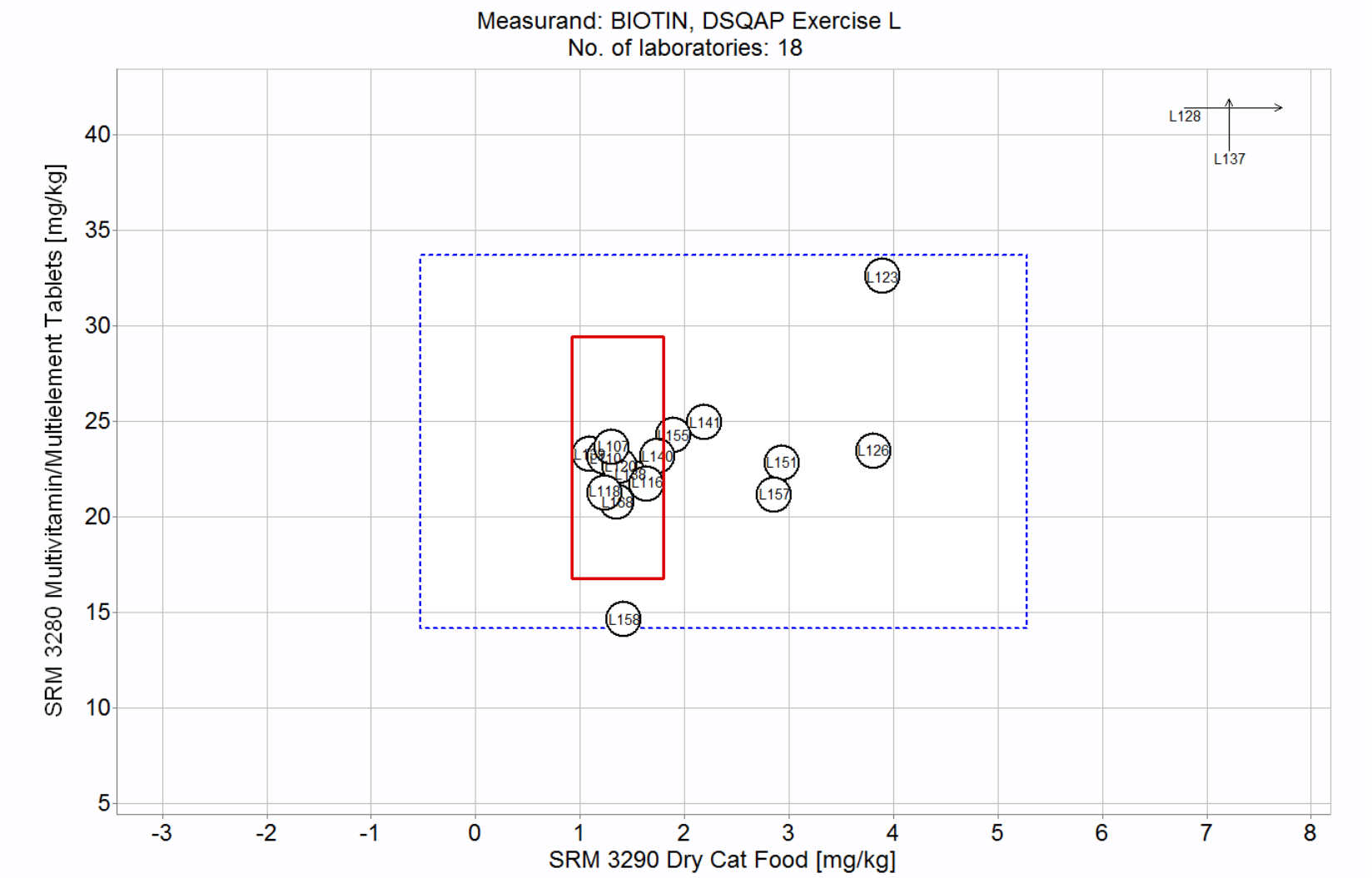

Figure 12. Biotin in SRM 3290 Dry Cat Food and SRM 3280 Multivitamin/Multielement Tablets (sample/sample comparison view). In this view, the individual laboratory results for one sample (cat food) are compared to the results for a second sample (multivitamin). The solid red box represents the target zone for the two samples, cat food (x-axis) and multivitamin (y-axis). The dotted blue box represents the consensus zone for cat food (x-axis) and multivitamin (y-axis).

XANTHOPHYLLS (LUTEIN AND ZEAXANTHIN) IN DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 2385 Slurried Spinach and SRM 3280 Multivitamin/ Multielement Tablets. Participants were asked to use in-house analytical methods to determine the mass fractions of lutein and zeaxanthin in each of the matrices and report values on an as-received basis.

Sample Information

Spinach. Participants were provided with one jar containing approximately 70 g of slurried spinach. The pureed spinach was blended, aliquotted, and sealed inside 2.5-oz. jars. Before use, participants were instructed to mix the contents of the jar thoroughly, and use a sample size of at least 1.5 g. Participants were asked to store the material under refrigeration, 0 °C to 4 °C, and to prepare three samples and report three values from the single jar provided. Approximate analyte levels were not reported to participants prior to the study. The certified value and uncertainty for total lutein in SRM 2385 was determined at NIST by LC-absorbance following solvent extraction with and without saponification, in combination with data from numerous collaborating laboratories. The certified value and uncertainty are reported in the table below on an as-received basis. The target value and uncertainty for zeaxanthin in SRM 2385 was determined at NIST by LC-absorbance following solvent extraction without saponification. The NIST-determined value and uncertainty are reported in the table below on an as-received basis.

Analyte	Certified Mass Fraction in SRM 2385 (mg/kg) (as-received basis)
Total Lutein	32.9 ± 6.5

Analyte	NIST-Determined Mass Fraction in SRM 2385 (mg/kg) (as-received basis)
Free Zeaxanthin	0.450 ± 0.080

Multivitamin/Multielement Tablets. Participants were provided with one bottle containing 30 multivitamin/multielement tablets. Before use, participants were instructed to grind all 30 tablets, mix the resulting powder thoroughly, and use a sample size of at least 2.0 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, prepare three samples, and report three values from the single bottle provided. Approximate analyte levels were not reported to participants prior to the study. The certified value and uncertainty for lutein in SRM 3280 was determined by LC-absorbance following solvent extraction, in combination with data from numerous collaborating laboratories. The certified value and uncertainty are reported in the table below on a dry-mass basis and after correction for moisture of the material (1.37 %). The target value and uncertainty for zeaxanthin in SRM 3280 was determined at NIST by LC-absorbance following solvent extraction without saponification. The NIST-determined value and uncertainty are reported in the table below on an as-received basis.

Analyte	Certified Mass Fraction in SRM 3280 (mg/kg) (dry-mass basis)	(as received basis)
Total Lutein	205 ± 50	202 ± 49

Analyte	NIST-Determined Mass Fraction in SRM 3280 (mg/kg) (as-received basis)
Total Zeaxanthin	5.4 ± 0.5

Study Results

- Forty laboratories enrolled in this exercise and received samples.
  - Thirteen laboratories reported results for lutein in the spinach sample (33 % participation). Seven laboratories reported results for zeaxanthin in the spinach sample (18 % participation). Thirteen and five laboratories were used, respectively, for calculation purposes, see Statistics, page 3.
  - Sixteen laboratories reported results for lutein in the multivitamin (40 % participation). Twelve laboratories reported results for zeaxanthin in the multivitamin (30 % participation). Sixteen and 12 laboratories were used, respectively, for calculation purposes, see Statistics, page 3.
- The consensus mean for lutein in the spinach was near the bottom of the target range with high between-laboratory variability (37 % RSD). The consensus mean for lutein in the multivitamin was within the target range with acceptable between-laboratory variability (15 % RSD).
- The consensus mean for zeaxanthin in the spinach was above the target range with extremely high between-laboratory variability (>100 % RSD). The consensus mean for zeaxanthin in the multivitamin was above the target range with high between-laboratory variability (30 % RSD).
- A majority of the laboratories reported using solvent extraction (86 %) as the sample preparation method. Some laboratories also reported using saponification (7 %), dilution (7 %), or no sample preparation technique (7 %).
- A majority of the laboratories reported using LC-absorbance (87 %) as their instrumental method for analysis. HPLC (7 %) and LC with a Diode Array Detector (LC-DAD, 7 %) were also reported by some laboratories.

Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- Care should be taken to minimize losses during the extraction process, during solvent evaporation, and by carefully washing down container walls with several rinses during each step to ensure complete dissolution of any residues.
- In general, laboratories reporting more vigorous extraction procedures, i.e. those using hexanes and longer extraction times, reported results closer to the target value.
- Since loss by photodecomposition is possible, care should be taken to prevent such losses (use of amber vials, aluminum foil, and/or reduced lighting).
- When using LC-absorbance, chromatographic coelutions may cause results to be biased high. This is particularly important if monitoring the absorbance in the UV where many

other compounds may also have chromophores. To avoid a high bias, more selective detectors (fluorescence, mass spectrometry) or chromatography with alternate selectivity may be used.

- When making calibration solutions make sure they are of known quality. These may need to be tested before running samples, which may include determination of purity by chromatographic and spectroscopic methods.
- If using an internal standard, the internal standard must behave similarly to the analyte of interest in extraction, chromatographic analysis, and detection.

Table 8. Individualized data summary table (NIST) for lutein and zeaxanthin in dietary supplements.

National Institute of Standards & Technology

Exercise L - October 2015 - Xanthophylls

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Lutein	Multivitamin	mg/kg	202	99		0.00	16	175	27	202	99
Lutein	Spinach	mg/kg	32.9	13.0		0.00	13	21.5	8.0	32.9	13.0
Zeaxanthin	Multivitamin	mg/kg	5.40	0.52		0.00	12	11.09	3.30	5.40	0.52
Zeaxanthin	Spinach	mg/kg	0.450	0.160		0.00	5	1.591	1.746	0.450	0.160

x_i Mean of reported values

s_i Standard deviation of reported values

Z'_{comm} Z'-score with respect to community consensus

Z_{NIST} Z-score with respect to NIST value

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 9. Data summary table for lutein in dietary supplements.

		Lutein									
		SRM 3280 MultivitaminTablets (mg/kg)					SRM 2385 Slurried Spinach (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				202	99				32.9	13.0
	L101										
	L102										
	L103										
	L104										
	L105										
	L107	166	164	165	165	1	18.6	18.8	18.6	18.7	0.1
	L110	167	171	160	166	6					
	L111	108	104	111	108	4					
	L112										
	L113	180	181	169	177	7	24.9	24.2	24.2	24.4	0.4
	L116										
	L117										
	L118	200	186	187	191	8	25.2	24.2	25.6	25.0	0.7
	L119	152	148	141	147	5	7.2	7.2	7.4	7.3	0.1
	L121	144	177	155	159	17	20.5	19.7	19.8	20.0	0.5
	L122										
	L123	269	251	263	261	9	8.6	9.8	8.7	9.0	0.7
	L128	174	154	175	168	12	21.0	21.0	21.0	21.0	0.0
	L130										
	L134										
	L137	166	156	173	165	8	19.3	19.5	24.1	21.0	2.7
	L138										
	L139	164	178	159	167	10	20.8	20.8	22.6	21.4	1.0
	L144										
	L145										
	L150										
	L158										
	L159										
	L166										
	L167	225	211	221	219	7	30.3	33.0	29.8	31.0	1.7
	L168	162	174	169	168	6	28.1	26.1	26.2	26.8	1.2
	L170	182	181	181	181	1					
	L171	975	1015	1035	1008	31	43.0	44.0	42.0	43.0	1.0
	L172										
	L177										
	L179	173	185	161	173	12	17.8	17.9	17.7	17.8	0.1
Community Results		Consensus Mean			175		Consensus Mean			21.5	
		Consensus Standard Deviation			27		Consensus Standard Deviation			8.0	
		Maximum			1008		Maximum			43.0	
		Minimum			108		Minimum			7.3	
		N			16		N			13	

Table 10. Data summary table for zeaxanthin in dietary supplements.

		Zeaxanthin									
		SRM 3280 MultivitaminTablets (mg/kg)					SRM 2385 Slurried Spinach (mg/kg)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				5.4	0.5				0.450	0.160
	L101										
	L102										
	L103										
	L104										
	L105										
	L107	13.4	13.7	13.7	13.6	0.2	0.638	0.646	0.644	0.643	0.004
	L110	13.0	13.4	12.5	13.0	0.5					
	L111	7.5	7.4	7.8	7.6	0.2					
	L112										
	L113	12.5	12.6	11.5	12.2	0.6	3.053	2.898	3.013	2.988	0.080
	L116										
	L117										
	L118	12.1	11.2	11.0	11.4	0.6	< 1.000	< 1.000	< 1.000		
	L121	8.9	8.3	10.1	9.1	0.9	0.459	0.397	0.453	0.436	0.034
	L122										
	L128	13.0	10.0	13.0	12.0	1.7					
	L130										
	L137	7.1	6.9	8.8	7.6	1.1					
	L138										
	L139	13.5	12.0	12.0	12.5	0.9	< 1.000	< 1.000	< 1.000		
	L144										
	L150										
	L158										
	L159										
	L160										
	L166										
	L167	31.4	29.6	33.4	31.5	1.9	3.900	3.400	3.300	3.533	0.321
	L168										
	L170	11.9	12.3	11.6	11.9	0.4					
	L172										
	L177										
	L179	4.7	4.7	4.6	4.7	0.1	0.350	0.360	0.360	0.357	0.006
Community Results		Consensus Mean			11.1		Consensus Mean			1.591	
		Consensus Standard Deviation			3.3		Consensus Standard Deviation			1.746	
		Maximum			31.5		Maximum			3.533	
		Minimum			4.7		Minimum			0.357	
		N			12		N			5	

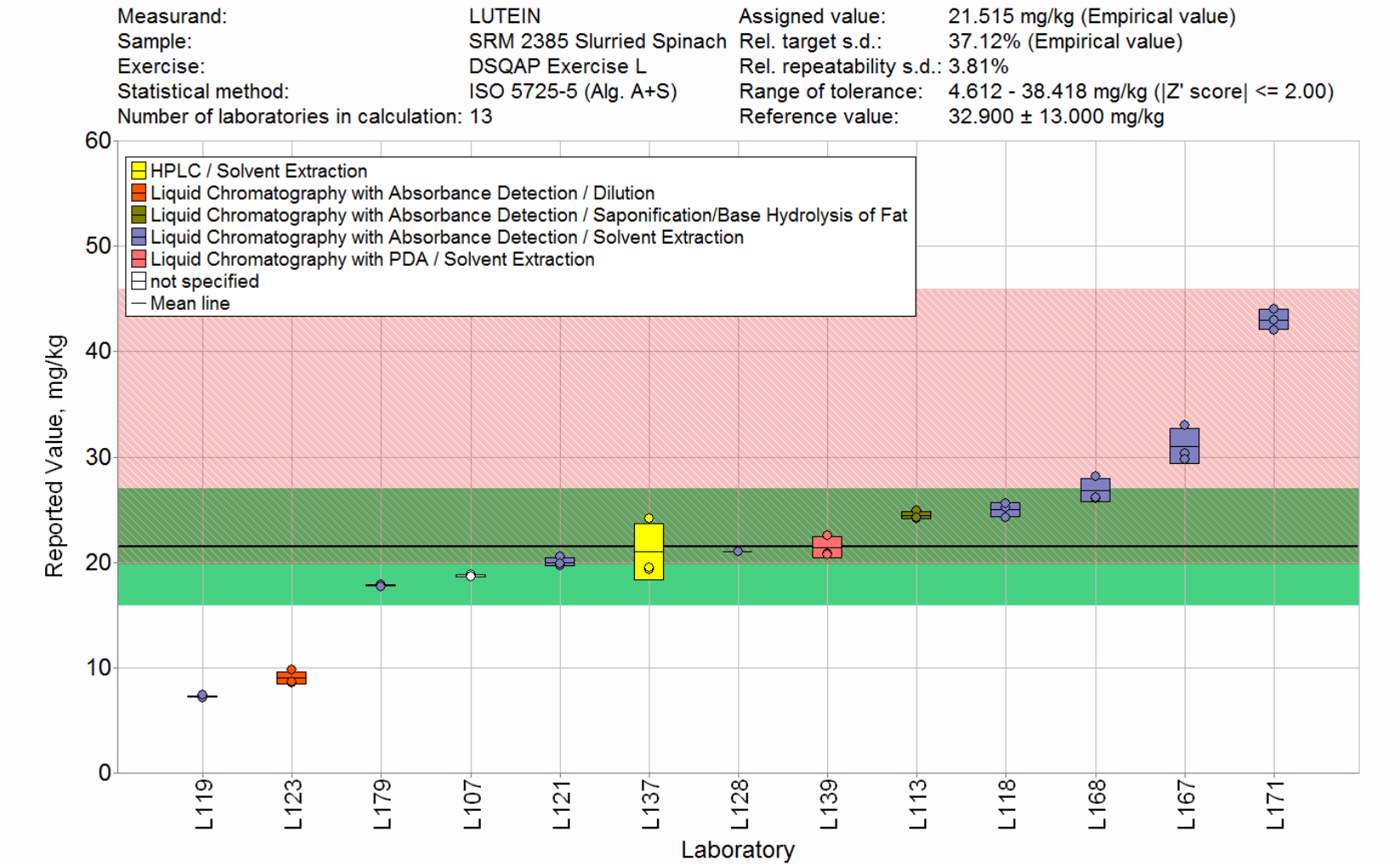

Figure 13. Lutein in SRM 2385 Slurried Spinach (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

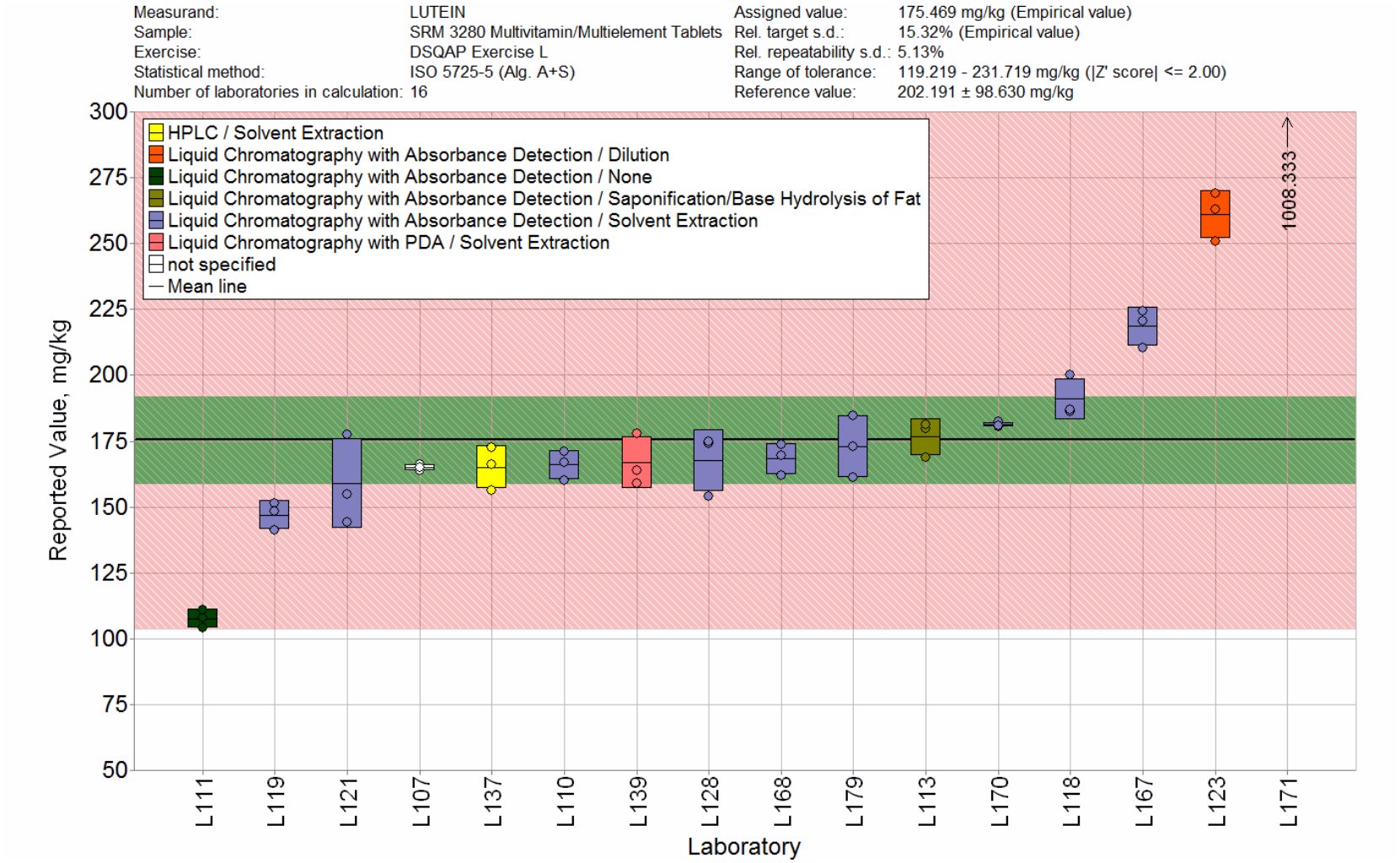

Figure 14. Lutein in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

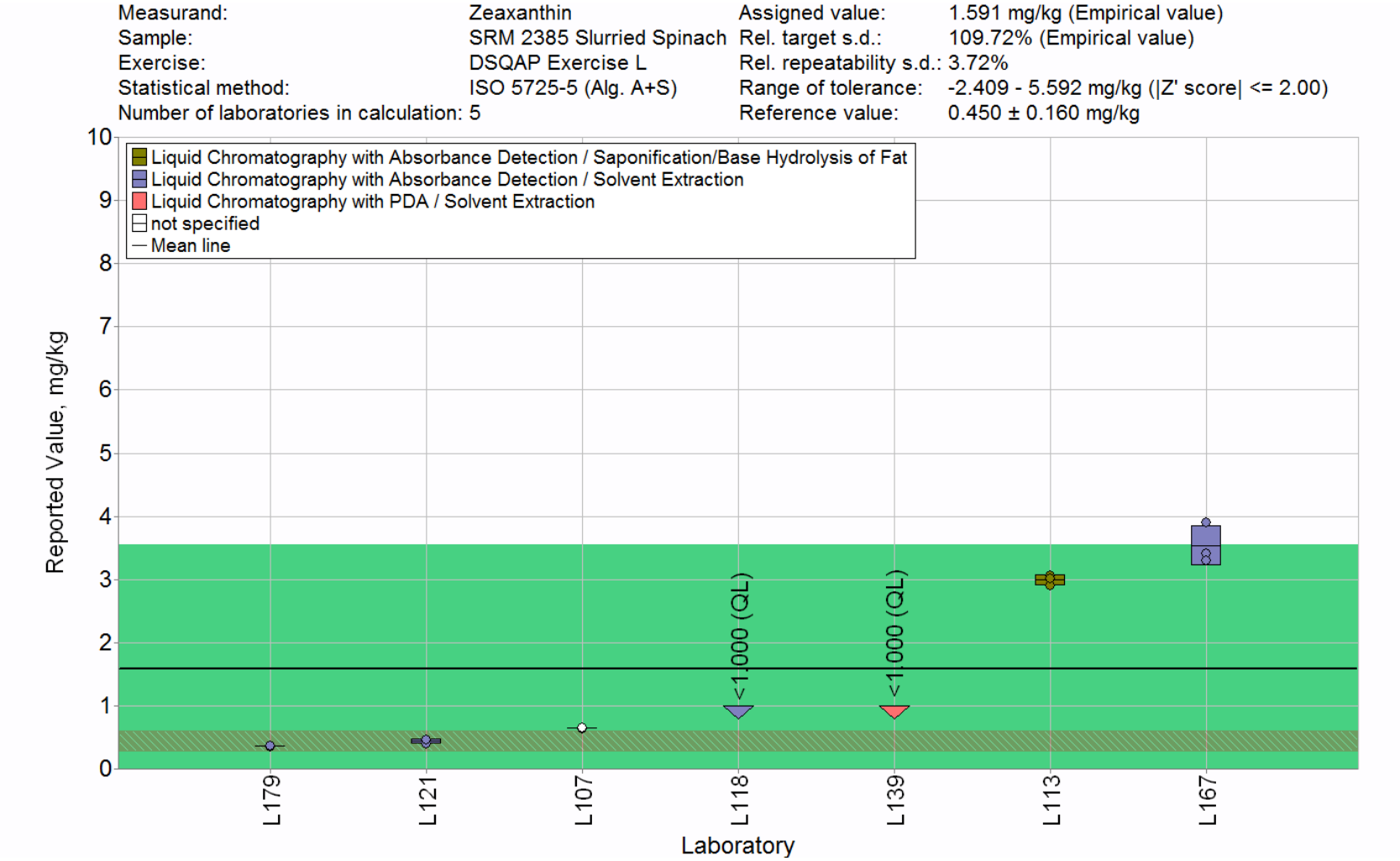

Figure 15. Zeaxanthin in SRM 2385 Slurried Spinach (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

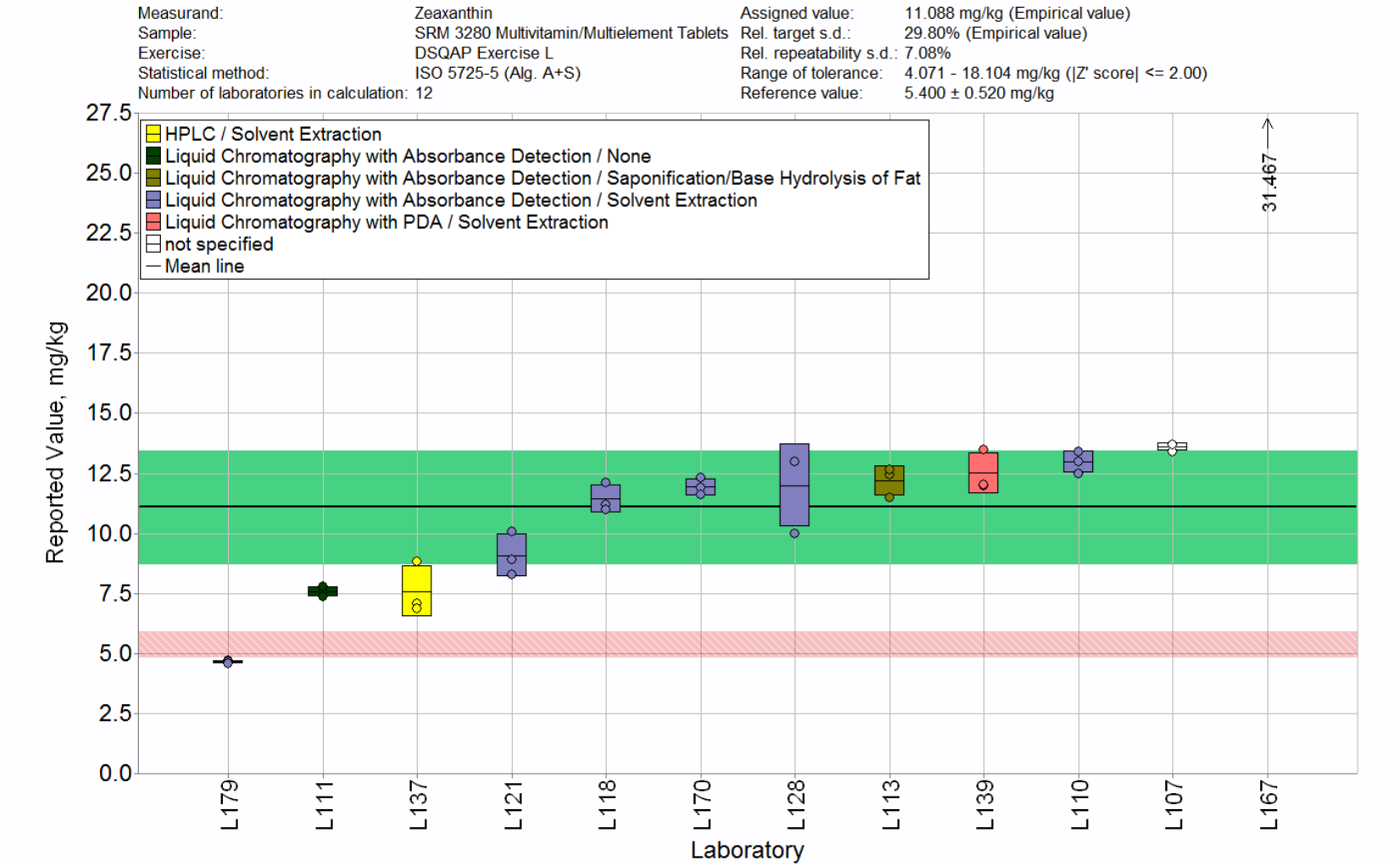

Figure 16. Zeaxanthin in SRM 3280 Multivitamin/Multielement Tablets (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

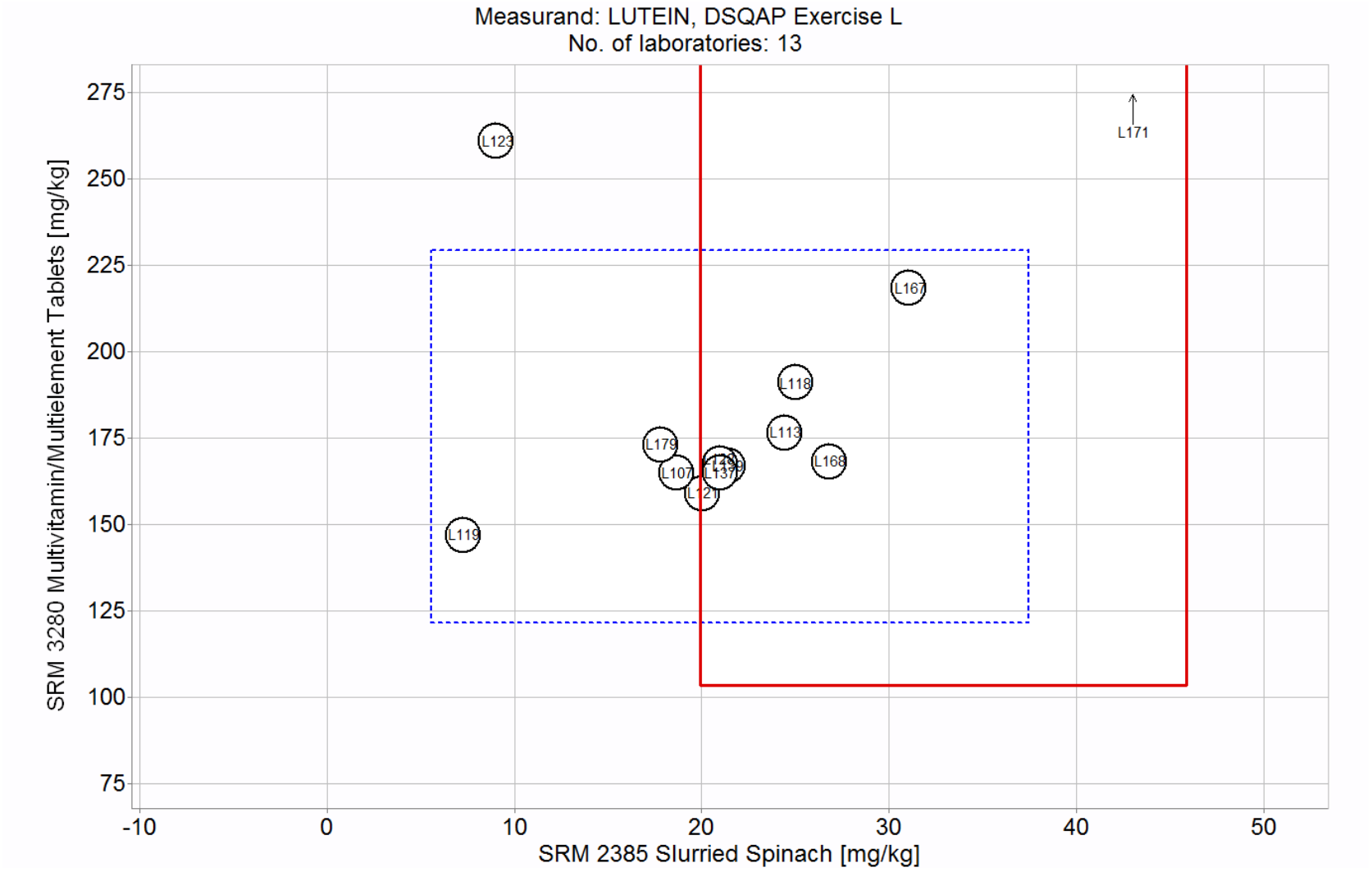

Figure 17. Lutein in SRM 2385 Slurried Spinach and SRM 3280 Multivitamin/Multielement Tablets (sample/sample comparison view). In this view, the individual laboratory results for one sample (spinach) are compared to the results for a second sample (multivitamin). The solid red box represents the target zone for the two samples, spinach (x-axis) and multivitamin (y-axis). The dotted blue box represents the consensus zone for spinach (x-axis) and multivitamin (y-axis).

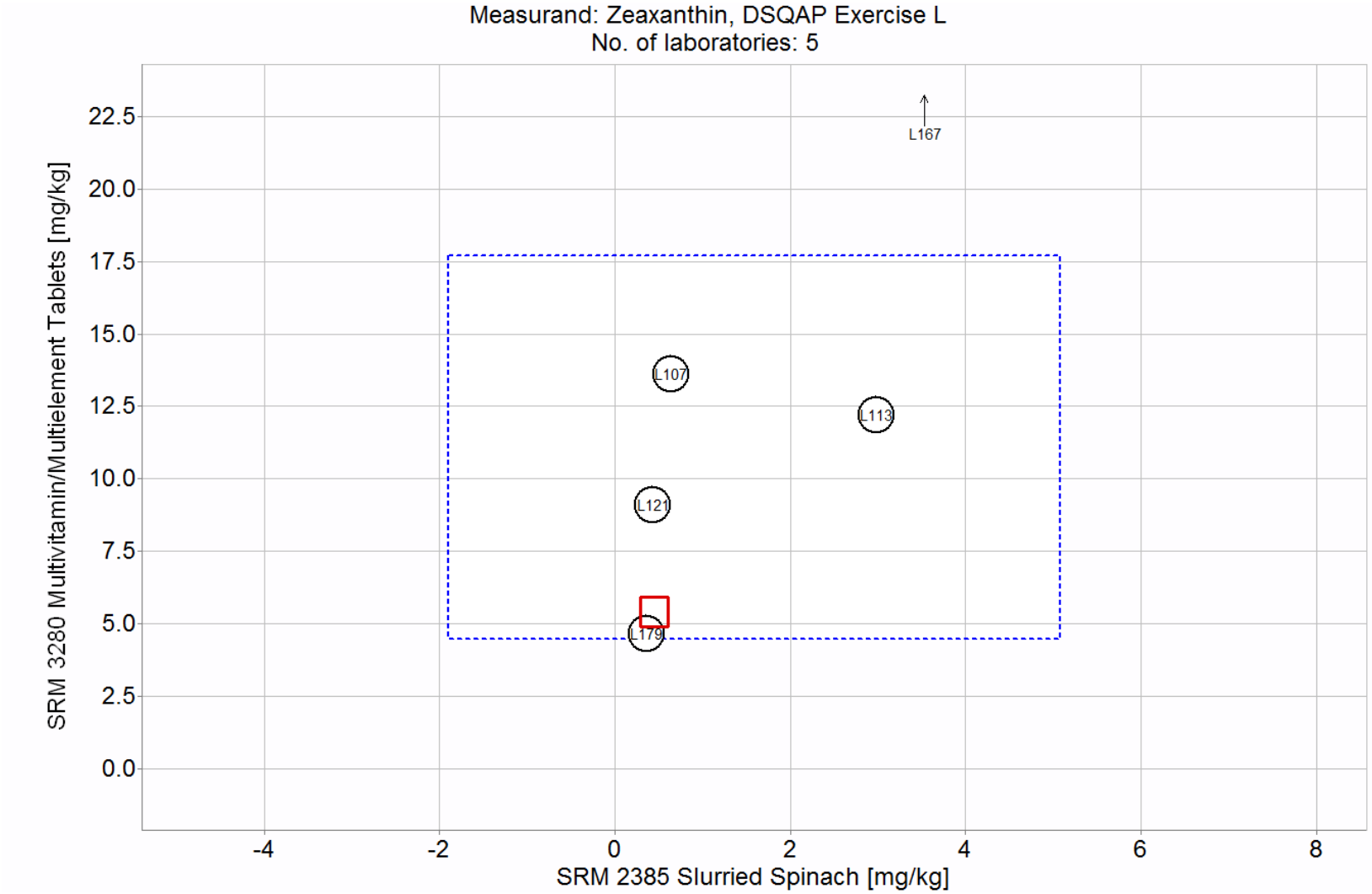

Figure 18. Zeaxanthin in SRM 2385 Slurried Spinach and SRM 3280 Multivitamin/Multielement Tablets (sample/sample comparison view). In this view, the individual laboratory results for one sample (spinach) are compared to the results for a second sample (multivitamin). The solid red box represents the target zone for the two samples, spinach (x-axis) and multivitamin (y-axis). The dotted blue box represents the consensus zone for spinach (x-axis) and multivitamin (y-axis).

FATTY ACIDS IN FISH OILS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil and SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil. Participants were asked to use in-house analytical methods to determine the mass fractions of six fatty acids (linoleic acid, α-linolenic acid, γ-linolenic acid, arachidonic acid, EPA, and DHA) in each of the matrices and report values on an as-received basis as fatty acid methyl esters (FAMEs).

Sample Information

Fish Oil 1. Participants were provided with three ampoules containing 1.2 mL of fish oil concentrate high in DHA. The fish oil was combined with mixed natural tocopherols (minimum 1 mg/g) as an antioxidant and ampouled under argon into 2 mL amber ampoules. Before use, participants were instructed to mix the contents of each ampoule thoroughly and use a sample size of at least 0.5 g. Participants were asked to store the material under refrigeration, 0 °C to 4 °C, and report a single value from each ampoule provided. Approximate analyte levels were not reported to participants prior to the study. The certified and reference values and uncertainties for fatty acids in SRM 3275-I were determined at NIST by gas chromatography with mass spectrometric detection (GC-MS) and GC with flame ionization detection (GC-FID). The certified and reference values and uncertainties are reported in the table below on an as-received basis.

Analyte	Certified and *Reference* Mass Fraction in SRM 3275-I (mg/g as FAME)
Linoleic Acid	2.31 ± 0.19
α-Linolenic Acid	*1.21* ± *0.05*
γ-Linolenic Acid	*0.344* ± *0.025*
Arachidonic Acid	*5.69* ± *0.19*
EPA	113 ± 12
DHA	*429* ± *15*

Fish Oil 2. Participants were provided with three ampoules containing 1.2 mL of fish oil concentrate containing 60 % long-chain omega-3 fatty acids. The fish oil was combined with mixed natural tocopherols (minimum 1 mg/g) as an antioxidant and ampouled under argon into 2 mL amber ampoules. Before use, participants were instructed to mix the contents of each ampoule thoroughly and use a sample size of at least 0.5 g. Participants were asked to store the material under refrigeration, 0 °C to 4 °C, and report a single value from each ampoule provided. Approximate analyte levels were not reported to participants prior to the study. The certified and reference values and uncertainties for fatty acids in SRM 3275-I were determined at NIST by gas chromatography with mass spectrometric detection (GC-MS) and GC with flame ionization detection (GC-FID). The certified and reference values and uncertainties are reported in the table below on an as-received basis.

Analyte	Certified and *Reference* Mass Fraction in SRM 3275-III (mg/g as FAME)
Linoleic Acid	13.49 ± 0.45
α-Linolenic Acid	*6.61* ± *0.31*
γ-Linolenic Acid	*1.771* ± *0.099*
Arachidonic Acid	not assigned
EPA	*154* ± *9*
DHA	*104* ± *5*

Study Results

- Forty laboratories enrolled in this exercise and received samples. Seventeen to twenty-three laboratories reported results (43 % to 58 % participation), depending on the analyte and matrix combination.
- In the first fish oil sample (SRM 3275-I, a concentrate high in DHA), the consensus means for all fatty acids were within the target ranges.
  - While within the target ranges, the consensus means for γ-linolenic acid, arachidonic acid, and DHA were near the upper bounds of the respective target ranges.
  - The between-laboratory variability for EPA and DHA was excellent at 10 % and 14 % RSD, respectively.
  - The variabilities for linoleic acid, α-linolenic acid, and arachidonic acid were acceptable at 25 % to 31 % RSD.
  - Results for γ-linolenic acid displayed very high between-laboratory variability (76 % RSD).
- In the second fish oil sample (SRM 3275-III, a concentrate containing 60 % long-chain omega-3 fatty acids), only the consensus means for α-linolenic acid, EPA, and DHA were within the target ranges.
  - While within the target ranges, the consensus means for α-linolenic acid and EPA were near the lower bounds of the respective target ranges. The consensus mean for DHA was near the upper bounds of the target range.
  - The consensus mean for linoleic acid was below the target range.
  - The consensus mean for γ-linolenic acid was above the target range.
  - No target range was provided for arachidonic acid.
  - The between-laboratory variability for EPA and DHA was acceptable at 22 % and 28 % RSD, respectively.
  - The variabilities for linoleic acid, α-linolenic acid, γ-linolenic acid, and arachidonic acid were high at 32 % to 57 % RSD.
- A majority of laboratories reported using saponification or base hydrolysis (41 %) or derivatization (36 %) for sample preparation. Other reported techniques included acid hydrolysis (9 %), solvent extraction (9 %), and dilution (5 %).
- A majority of laboratories reported using gas chromatography with flame ionization detection (GC-FID) as their analytical method of analysis (91 %). GC with mass spectrometric detection (GC-MS) was also reported (9 %).

Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- With a small number of laboratories reporting data for these fatty acids, and a majority reporting use of the same or very similar methods, drawing extensive technical conclusions is difficult.
- Participants were asked to report concentrations for fatty acids as fatty acid methyl esters (FAMEs). In this case, FAMEs should be used as calibrants or non-esterified fatty acids should be carried through the entire sample preparation procedure (hydrolysis and derivatization) to improve quantitation.
- Knowledge of calibrant response when carried through the derivatization procedure is necessary. For example, at NIST, calibrants for EPA and DPA give response factors of 1.3 and 1.6, respectively, corresponding to 30 % or 60 % low bias in the quantitation of these compounds if not considered.
- Similarly, for those laboratories using GC-MS, quantitation for some compounds may be inaccurate as a result of non-unity response factors from EI fragmentation.

Table 11. Individualized data summary table (NIST) for fatty acids in fish oils.

National Institute of Standards & Technology

Exercise L - October 2015 - Fatty Acids

	Lab Code:	**NIST**	**1. Your Results**				**2. Community Results**			**3. Target**	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Linoleic Acid	Fish Oil 1	mg/g	2.31	0.38		0.00	20	2.24	0.57	2.31	0.38
Linoleic Acid	Fish Oil 2	mg/g	13.49	0.90		0.00	20	11.28	3.62	13.49	0.90
α-Linolenic Acid	Fish Oil 1	mg/g	1.21	0.10		0.00	19	1.26	0.35	1.21	0.10
α-Linolenic Acid	Fish Oil 2	mg/g	6.61	0.62		0.00	20	6.31	2.31	6.61	0.62
γ-Linolenic Acid	Fish Oil 1	mg/g	0.344	0.050		0.00	14	0.389	0.297	0.344	0.050
γ-Linolenic Acid	Fish Oil 2	mg/g	1.77	0.20		0.00	18	2.12	1.21	1.77	0.20
Arachidonic Acid	Fish Oil 1	mg/g	5.69	0.38		0.00	18	6.02	1.85	5.69	0.38
Arachidonic Acid	Fish Oil 2	mg/g					18	11.0	4.4		
EPA	Fish Oil 1	mg/g	113	24		0.00	22	109	11	113	24
EPA	Fish Oil 2	mg/g	154	18		0.00	21	145	32	154	18
DHA	Fish Oil 1	mg/g	429	30		0.00	22	448	63	429	30
DHA	Fish Oil 2	mg/g	104	10		0.00	21	109	31	104	10

x_i Mean of reported values

s_i Standard deviation of reported values

Z'_{comm} Z'-score with respect to community consensus

Z_{NIST} Z-score with respect to NIST value

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 12. Data summary table for linoleic acid in fish oils.

Linoleic Acid									
SRM 3275-I Fish Oil (mg/g)					SRM 3275-III Fish Oil (mg/g)				
A	B	C	Avg	SD	A	B	C	Avg	SD
			2.31	0.38				13.49	0.90
2.37	2.33	2.30	2.33	0.04	12.20	12.20	12.20	12.20	0.00
2.43	2.55	< 1.00	2.49	0.08	2.43	2.50	2.72	2.55	0.15
2.91	2.89	2.87	2.89	0.02	16.10	16.20	15.95	16.08	0.13
2.14	2.16	2.15	2.15	0.01	11.85	11.87	11.87	11.86	0.01
3.76	3.60	3.68	3.68	0.08	18.53	18.09	18.31	18.31	0.22
					11.80	11.80	11.90	11.83	0.06
2.07	2.09	2.00	2.05	0.05					
1.10	0.93	0.96	1.00	0.09	9.14	9.37	9.40	9.30	0.14
2.04	2.08	2.08	2.07	0.02	11.75	11.50	11.61	11.62	0.13
3.00	3.00	3.00	3.00	0.00	15.00	15.00	14.00	14.67	0.58
0.99	0.56	0.63	0.73	0.23	4.37	4.29	3.40	4.02	0.54
2.15	2.14	2.23	2.17	0.05	10.11	10.37	10.47	10.32	0.19
1.50	1.50	1.40	1.47	0.06	10.60	10.60	10.60	10.60	0.00
2.19	2.10	2.10	2.13	0.05	12.03	12.14	12.24	12.14	0.11
3.07	3.57	3.63	3.42	0.31	13.50	13.90	14.00	13.80	0.26
2.29	2.29	2.57	2.38	0.16	2.37	2.34	2.62	2.44	0.15
	2.20	2.20	2.20	0.00		11.90	12.00	11.95	0.07
2.11	2.14	2.12	2.12	0.02	11.90	12.00	11.90	11.93	0.06
2.09	2.12	2.15	2.12	0.03	13.98	14.05	14.09	14.04	0.06
2.00	2.10	2.04	2.05	0.05	11.87	11.83	11.97	11.89	0.07
2.20	2.20	2.20	2.20	0.00	6.90	6.30	7.90	7.03	0.81
Consensus Mean			2.24		Consensus Mean			11.28	
Consensus Standard Deviation			0.57		Consensus Standard Deviation			3.62	
Maximum			3.68		Maximum			18.31	
Minimum			0.73		Minimum			2.44	
N			20		N			20	

Table 13. Data summary table for α-linolenic acid in fish oils.

		α-Linolenic Acid									
		SRM 3275-I Fish Oil (mg/g)					SRM 3275-III Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				1.21	0.10				6.61	0.62
	L103										
	L104										
	L105										
	L107	1.25	1.28	1.27	1.27	0.02	7.02	6.97	6.95	6.98	0.04
	L110										
	L111	< 1.00	< 1.00	< 1.00			1.89	1.60	< 1.00	1.75	0.21
	L112										
	L113	1.71	1.70	1.69	1.70	0.01	9.25	9.30	9.14	9.23	0.08
	L114										
	L116	1.20	1.21	1.20	1.20	0.01	6.68	6.68	6.69	6.68	0.01
	L117										
	L121	1.10	1.10	1.30	1.17	0.12	7.00	6.90	7.00	6.97	0.06
	L125	1.21	1.23	1.19	1.21	0.02					
	L129										
	L130										
	L131	0.21	0.20	0.22	0.21	0.01	3.98	3.96	4.51	4.15	0.31
	L133										
	L134										
	L135	1.15	0.80	1.23	1.06	0.23	7.06	6.99	7.03	7.03	0.04
	L136	2.00	2.00	2.00	2.00	0.00	9.00	9.30	9.00	9.10	0.17
	L137						2.40	2.40	1.78	2.19	0.36
	L139	1.24	1.24	1.35	1.28	0.06	6.40	6.79	6.64	6.61	0.20
	L140										
	L144										
	L146	0.60	0.60	0.70	0.63	0.06	5.90	6.00	5.90	5.93	0.06
	L149	1.16	1.13	1.16	1.15	0.02	6.45	6.64	6.38	6.49	0.13
	L151										
	L152	1.66	1.71	1.75	1.71	0.05	7.40	7.55	7.72	7.56	0.16
	L155										
	L157										
	L158	1.33	1.33	1.29	1.32	0.02	1.28	1.20	1.21	1.23	0.04
	L159										
	L160		1.40	1.40	1.40	0.00		7.00	7.00	7.00	0.00
	L164										
	L165	1.75	1.79	1.76	1.77	0.02	10.60	10.80	10.80	10.73	0.12
	L168	1.13	1.21	1.16	1.17	0.04	7.02	7.15	7.09	7.09	0.07
	L170	0.90	0.97	0.94	0.94	0.04	6.22	6.15	6.24	6.20	0.05
	L172										
	L176										
	L177										
	L179	1.20	1.10	1.20	1.17	0.06	4.00	3.60	4.50	4.03	0.45
	L182	1.21	1.20	1.27	1.23	0.04	6.72	6.84	6.74	6.77	0.06
Community Results		Consensus Mean			1.26		Consensus Mean			6.31	
		Consensus Standard Deviation			0.35		Consensus Standard Deviation			2.31	
		Maximum			2.00		Maximum			10.73	
		Minimum			0.21		Minimum			1.23	
		N			19		N			20	

Table 14. Data summary table for γ-linolenic acid in fish oils.

		γ-Linolenic Acid									
		SRM 3275-I Fish Oil (mg/g)					SRM 3275-III Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.344	0.050				1.77	0.20
	L103										
	L104										
	L105										
	L107	< 0.550	< 0.550	< 0.550			1.79	1.99	1.95	1.91	0.11
	L110										
	L111	< 1.000	< 1.000	< 1.000			6.32	< 1.000	< 1.000	6.32	
	L112										
	L113	0.466	0.446	0.457	0.456	0.010	2.87	2.89	2.84	2.87	0.03
	L114										
	L116	0.290	0.260	0.270	0.273	0.015	1.90	1.90	1.88	1.89	0.01
	L117										
	L121						2.40	2.50	2.40	2.43	0.06
	L125	0.242	0.265	0.251	0.253	0.012					
	L129										
	L130										
	L131	0.060	0.059	0.060	0.060	0.001	3.09	3.10	3.34	3.18	0.14
	L133										
	L134										
	L135	0.001	0.001	0.001	0.001	0.000	1.64	1.68	1.53	1.62	0.08
	L136	13.000	13.000	13.000	13.000	0.000	11.00	9.00	4.00	8.00	3.61
	L137						0.29	0.44	0.29	0.34	0.09
	L139	0.110	0.120	0.110	0.113	0.006	0.33	0.37	0.33	0.34	0.02
	L140										
	L144										
	L146	2.900	2.700	2.800	2.800	0.100	3.10	3.20	3.20	3.17	0.06
	L149	0.275	0.367	0.308	0.317	0.047	1.79	1.84	1.84	1.82	0.03
	L151										
	L152	0.470	0.620	0.610	0.567	0.084	2.44	2.35	2.35	2.38	0.05
	L155										
	L157										
	L158	0.460	0.580	0.960	0.667	0.261	0.40	0.68	0.66	0.58	0.16
	L159										
	L160		0.370	0.350	0.360	0.014		2.00	2.00	2.00	0.00
	L164										
	L165	< 0.100	< 0.100	< 0.100			2.46	2.48	2.45	2.46	0.02
	L168	0.390	0.420	0.430	0.413	0.021	1.82	1.93	1.89	1.88	0.06
	L170										
	L172										
	L176										
	L177										
	L179	0.300	0.300	0.300	0.300	0.000	1.30	1.20	1.50	1.33	0.15
	L182										
Community Results		Consensus Mean			0.389		Consensus Mean			2.12	
		Consensus Standard Deviation			0.297		Consensus Standard Deviation			1.21	
		Maximum			13.000		Maximum			8.00	
		Minimum			0.001		Minimum			0.34	
		N			14		N			18	

Table 15. Data summary table for arachidonic acid in fish oils.

		Arachidonic Acid									
		SRM 3275-I Fish Oil (mg/g)					SRM 3275-III Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				5.69	0.38					
	L103										
	L104										
	L105										
	L107	5.94	5.89	5.87	5.90	0.04	13.20	13.10	13.20	13.17	0.06
	L110										
	L111	7.26	6.68	7.01	6.98	0.29	6.30	6.93	6.69	6.64	0.32
	L112										
	L113	8.32	8.35	8.28	8.31	0.04	17.33	17.45	17.19	17.32	0.13
	L114										
	L116	6.07	6.10	6.14	6.10	0.04	12.89	12.85	12.81	12.85	0.04
	L117										
	L119	5.55	5.58	5.56	5.56	0.02	12.88	12.73	12.80	12.80	0.08
	L121						13.10	13.00	13.00	13.03	0.06
	L125	11.80	12.00	11.70	11.83	0.15					
	L129										
	L130										
	L131	3.24	3.19	2.88	3.10	0.20	7.82	8.36	9.17	8.45	0.68
	L134										
	L135	5.33	5.42	5.49	5.41	0.08	12.49	12.31	12.49	12.43	0.10
	L136	1.00	1.00	1.00	1.00	0.00	3.00	11.00	10.00	8.00	4.36
	L137	2.32	1.97	1.95	2.08	0.21	4.58	4.73	3.57	4.29	0.63
	L139	6.33	6.20	6.08	6.20	0.13	14.72	15.06	15.42	15.07	0.35
	L140										
	L144										
	L146	7.00	7.10	7.00	7.03	0.06	13.90	13.80	13.80	13.83	0.06
	L149	6.97	6.68	7.05	6.90	0.19	13.23	13.09	13.47	13.26	0.19
	L151										
	L152	7.84	8.18	8.24	8.09	0.22	14.80	15.30	15.30	15.13	0.29
	L155										
	L157										
	L158	7.00	6.74	6.65	6.80	0.18	6.96	6.75	6.59	6.77	0.19
	L159										
	L164										
	L165										
	L168	5.02	5.20	5.16	5.13	0.09	0.98	0.89	0.93	0.93	0.05
	L170	5.59	5.77	5.65	5.67	0.09	13.04	12.87	13.08	13.00	0.11
	L172										
	L176										
	L177										
	L179	5.90	5.70	5.70	5.77	0.12	7.70	7.00	8.80	7.83	0.91
	L182										
Community Results		Consensus Mean			6.02		Consensus Mean			11.02	
		Consensus Standard Deviation			1.85		Consensus Standard Deviation			4.42	
		Maximum			11.83		Maximum			17.32	
		Minimum			1.00		Minimum			0.93	
		N			18		N			18	

Table 16. Data summary table for EPA in fish oils.

		EPA									
		SRM 3275-I Fish Oil (mg/g)					SRM 3275-III Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				113	24				154	18
	L103										
	L104										
	L105										
	L107	107	107	108	107	1	155	155	154	155	1
	L110										
	L111	110	118	112	113	4	111	113	114	113	2
	L112										
	L113	142	144	142	143	1	206	207	205	206	1
	L114										
	L116	112	112	112	112	0	157	158	158	158	1
	L117										
	L119	110	110	110	110	0	157	156	157	157	1
	L121	112	110	109	110	2	153	152	151	152	1
	L125	106	107	104	106	2					
	L129										
	L130										
	L131	82	73	72	76	6	108	107	118	111	6
	L133	114	115		115	1	111	112		112	1
	L134										
	L135	99	102	104	102	3	148	149	147	148	1
	L136	146	145	146	146	1	206	214	201	207	7
	L137	45	37	37	40	5	58	59	47	55	7
	L139	113	106	114	111	4	169	167	172	169	3
	L140										
	L144										
	L146	103	103	101	102	1	146	146	145	146	1
	L149	100	100	100	100	0	146	147	148	147	1
	L151										
	L152	149	146	146	147	2	172	175	176	174	2
	L155										
	L157										
	L158	106	106	106	106	0	106	106	106	106	0
	L159										
	L160	92	95	94	94	2	136	137	135	136	1
	L164										
	L165	110	111	109	110	1	159	160	160	160	1
	L168	120	125	121	122	3	161	164	163	163	2
	L170	104	108	106	106	2	158	156	158	157	1
	L171										
	L172										
	L176										
	L177										
	L179	110	107	107			95	87	107		
	L182	107	107	109	108	1	153	155	153	154	1
Community Results		Consensus Mean			109		Consensus Mean			145	
		Consensus Standard Deviation			11		Consensus Standard Deviation			32	
		Maximum			147		Maximum			207	
		Minimum			40		Minimum			55	
		N			22		N			21	

Table 17. Data summary table for DHA in fish oils.

		DHA									
		SRM 3275-I Fish Oil (mg/g)					SRM 3275-III Fish Oil (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				429	30				104	10
	L103										
	L104										
	L105										
	L107	428	427	430	428	2	102	102	102	102	0
	L110										
	L111	438	474	450	454	18	441	443	452	445	6
	L112										
	L113	569	574	568	570	3	136	137	135	136	1
	L114										
	L116	500	502	500	501	1	108	108	109	108	1
	L117										
	L119	427	430	429	429	2	103	101	102	102	1
	L121	447	444	438	443	5	101	100	99	100	1
	L125	411	419	415	415	4					
	L129										
	L130										
	L131	265	232	243	247	17	55	54	60	56	3
	L133	525	528		527	2	492	493		493	1
	L134										
	L135	394	401	411	402	9	97	96	97	97	1
	L136	596	596	593	595	2	143	148	138	143	5
	L137	206	171	175	184	19	36	36	29	34	4
	L139	472	445	474	464	16	113	115	116	115	2
	L140										
	L144										
	L146	407	409	405	407	2	98	99	98	98	1
	L149	390	389	392	390	2	94	94	95	94	1
	L151										
	L152	558	577	575	570	10	111	113	114	113	2
	L155										
	L157										
	L158	427	424	424	425	2	428	424	423	425	3
	L159										
	L160	410	420	418	416	5	99	101	99	100	1
	L164										
	L165	500	498	489	496	6	112	112	111	112	1
	L168	434	440	438	437	3	110	113	112	112	2
	L170	437	445	439	440	4	104	103	104	104	1
	L171										
	L172										
	L176										
	L177										
	L179	483	467	470			62	57	71		
	L182	430	430	434	431	2	101	103	101	102	1
Community Results		Consensus Mean			448		Consensus Mean			109	
		Consensus Standard Deviation			63		Consensus Standard Deviation			31	
		Maximum			595		Maximum			493	
		Minimum			184		Minimum			34	
		N			22		N			21	

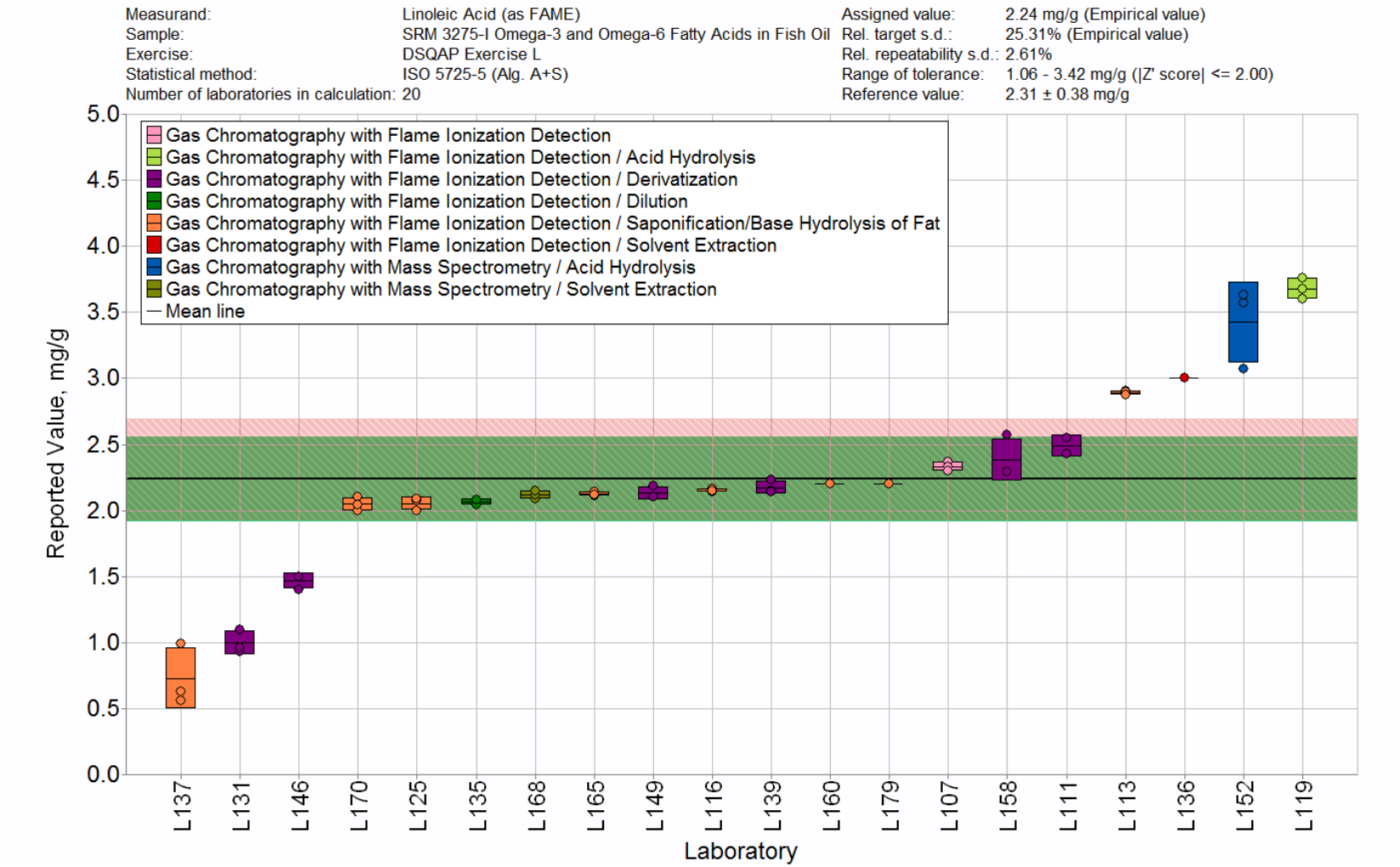

Figure 19. Linoleic acid in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

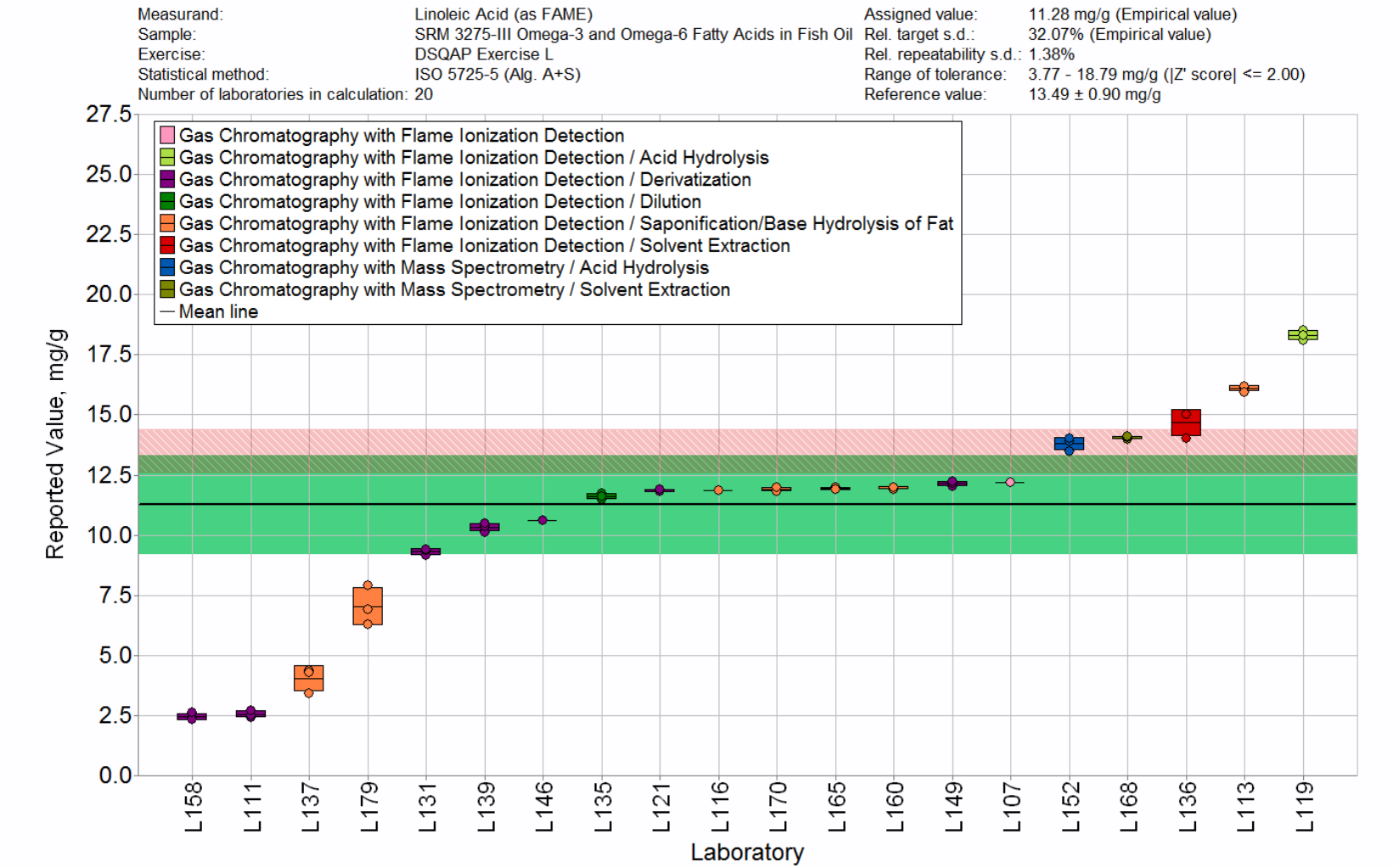

Figure 20. Linoleic acid in SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

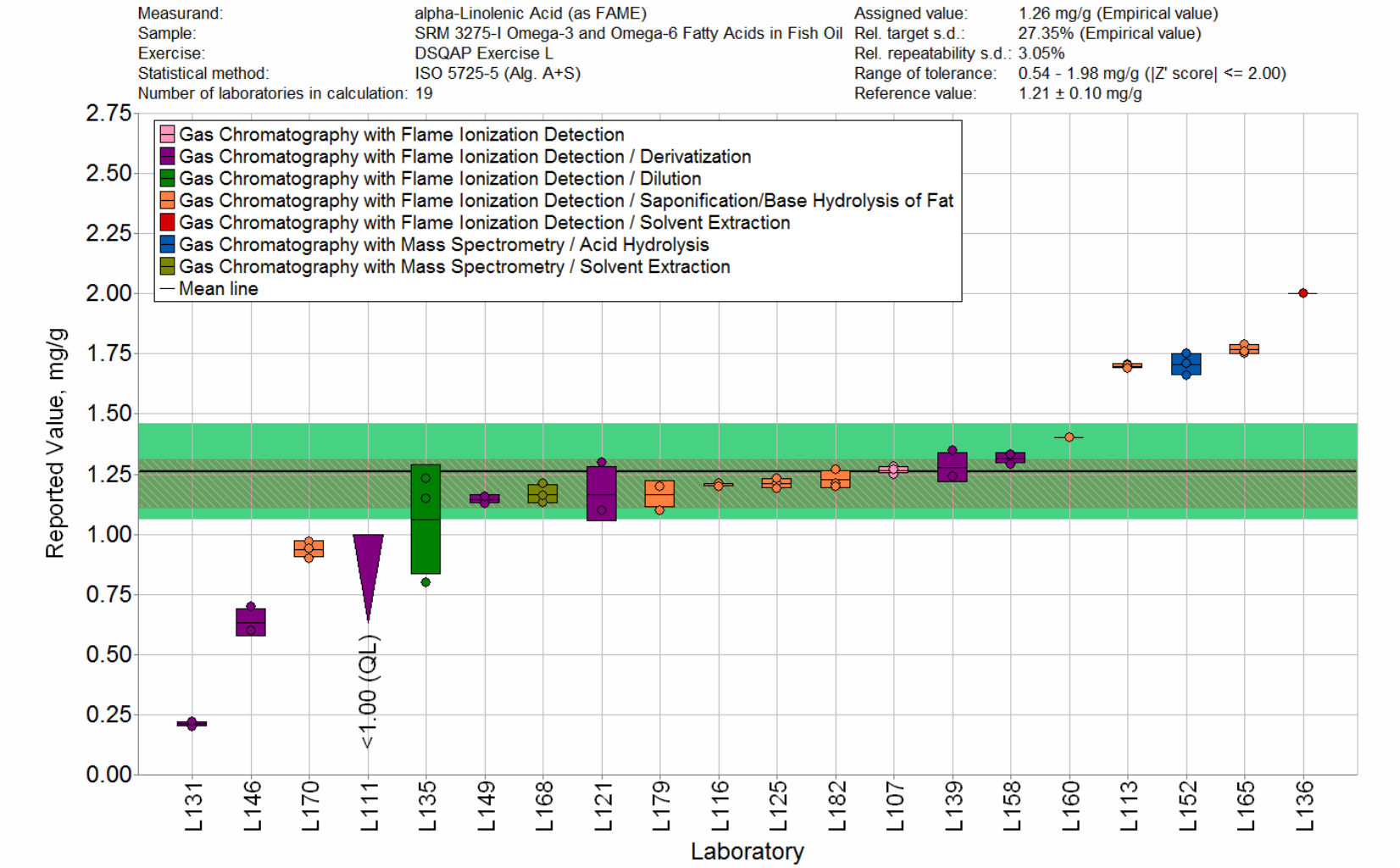

Figure 21. α-Linolenic acid in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

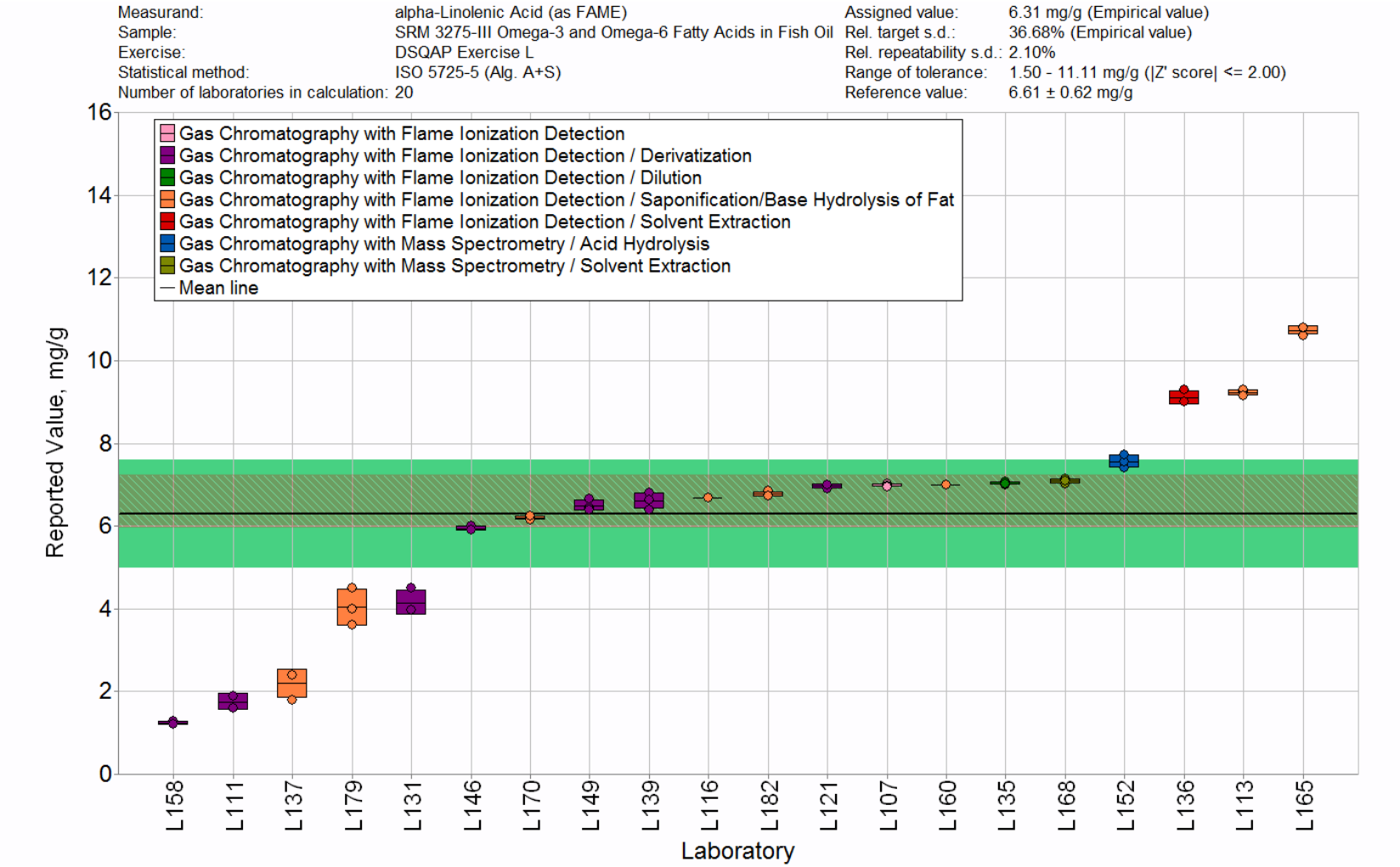

Figure 22. α-Linolenic acid in SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

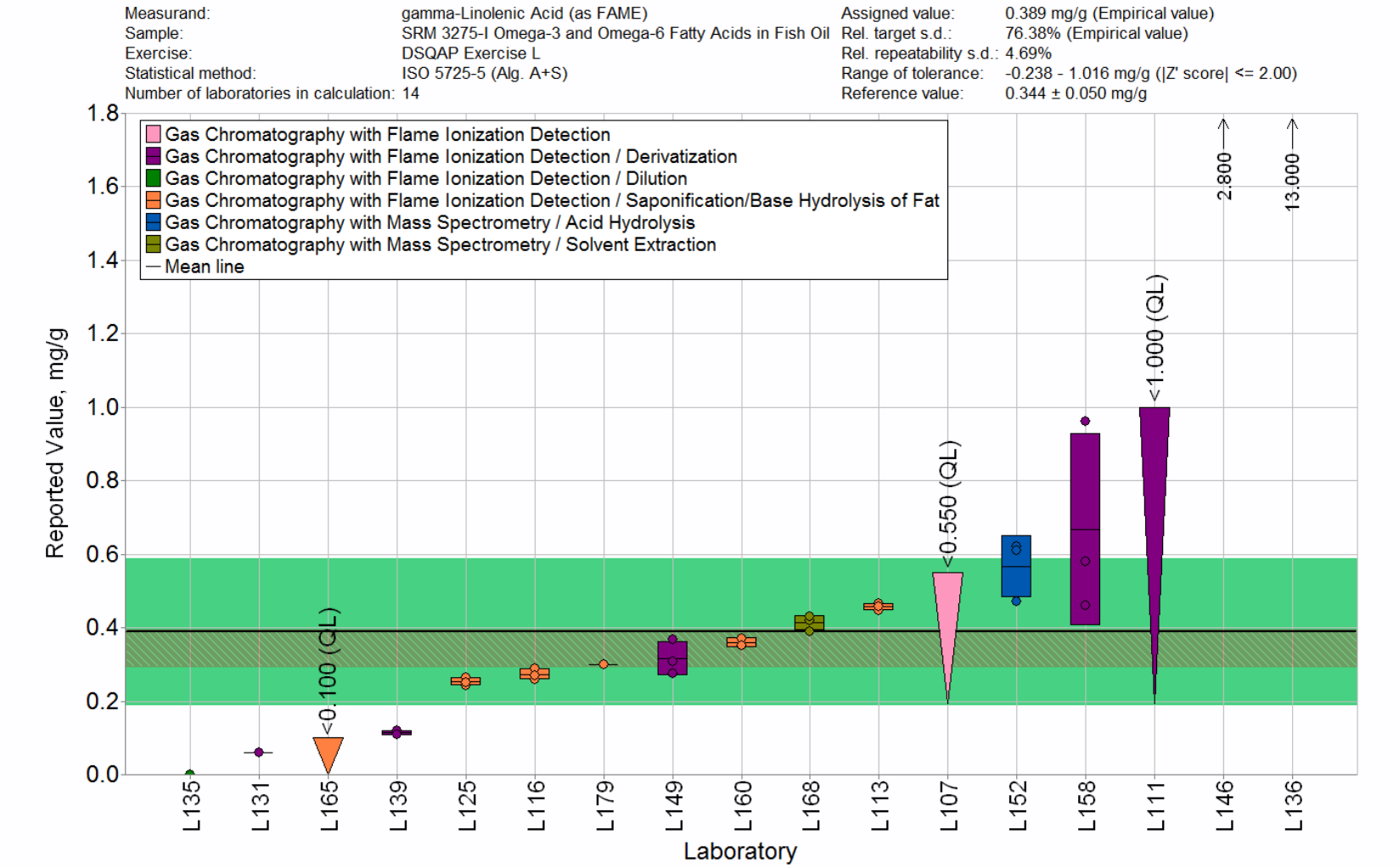

Figure 23. γ-Linolenic acid in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

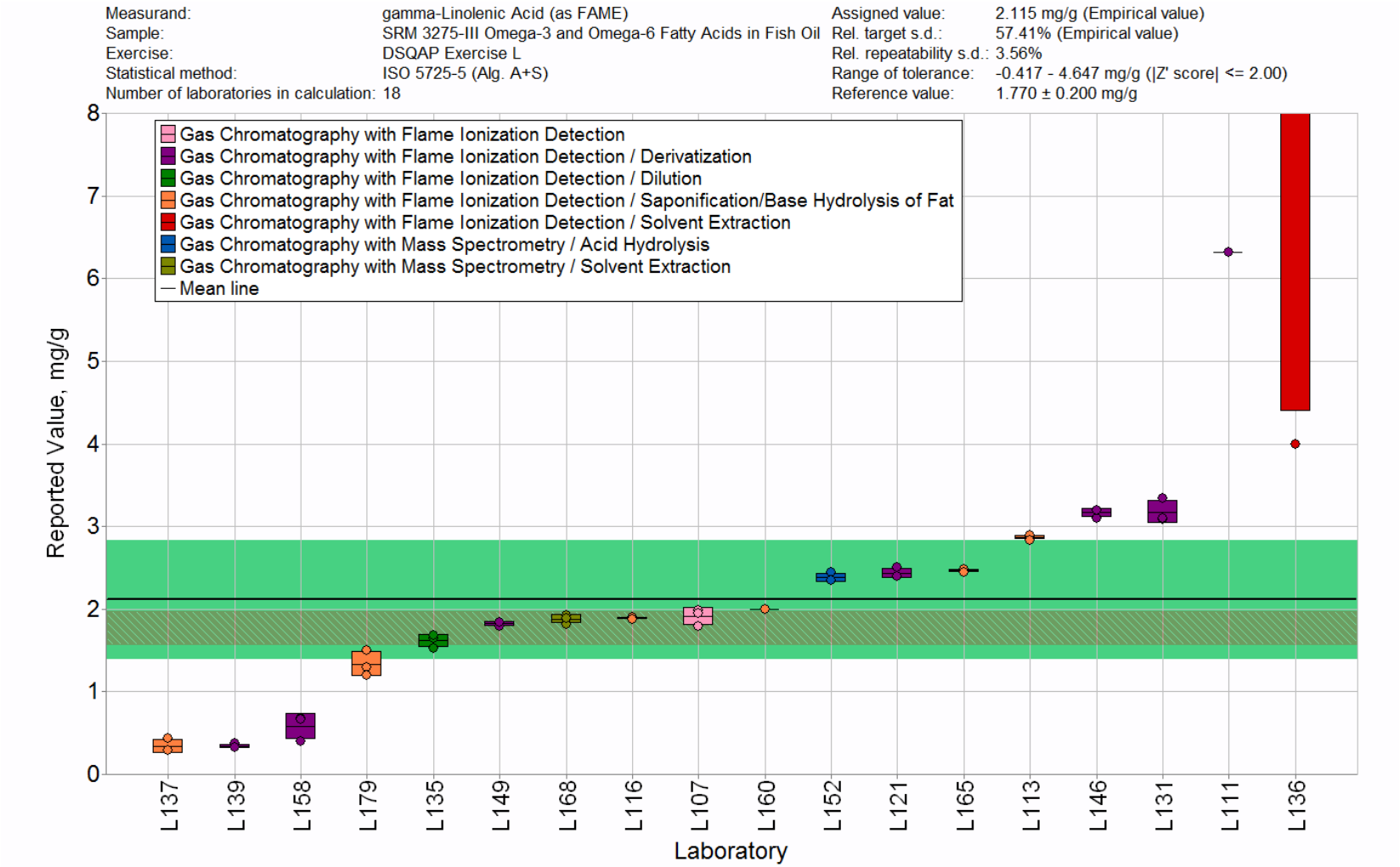

Figure 24. γ-Linolenic acid in SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

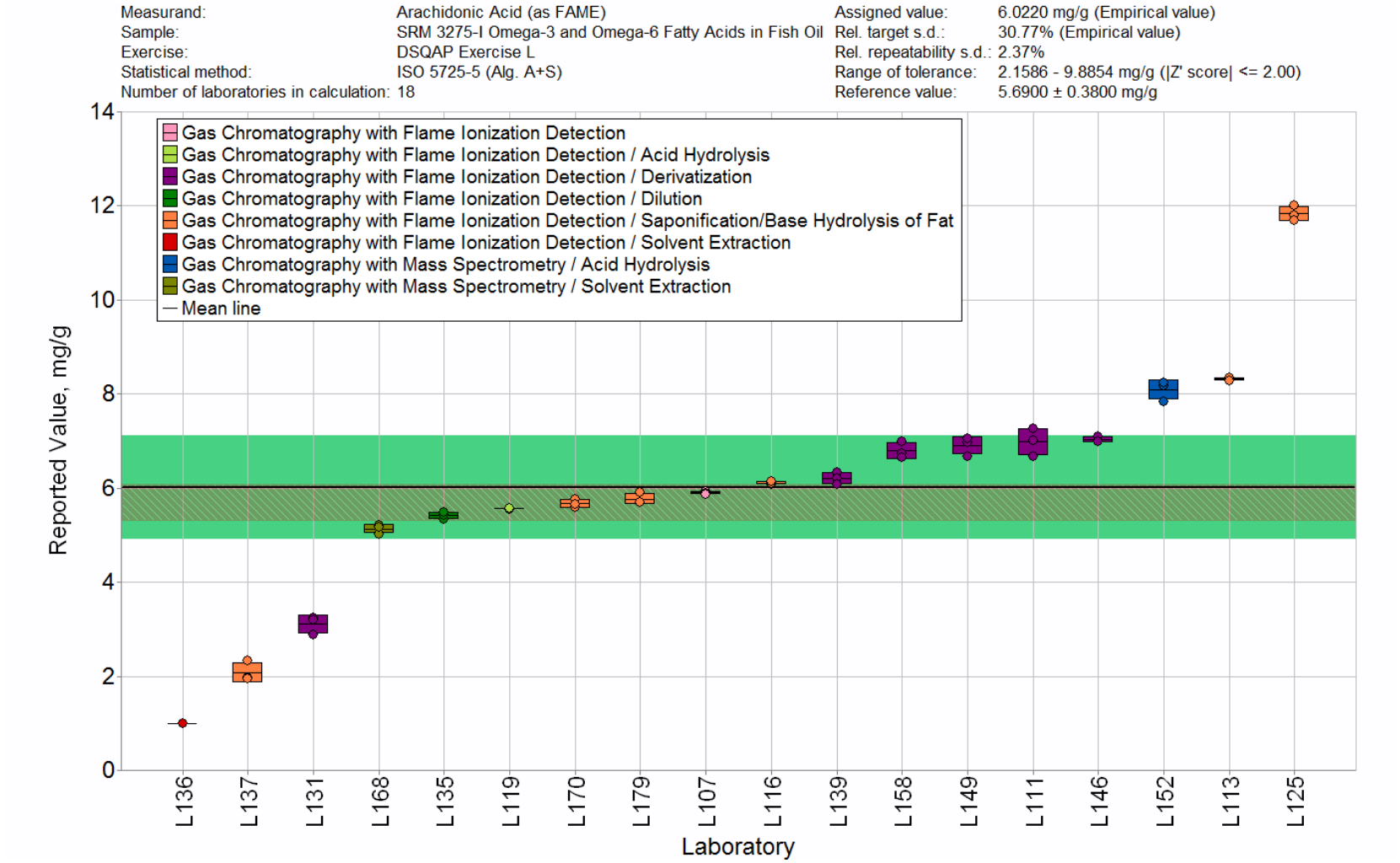

Figure 25. Arachidonic acid in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

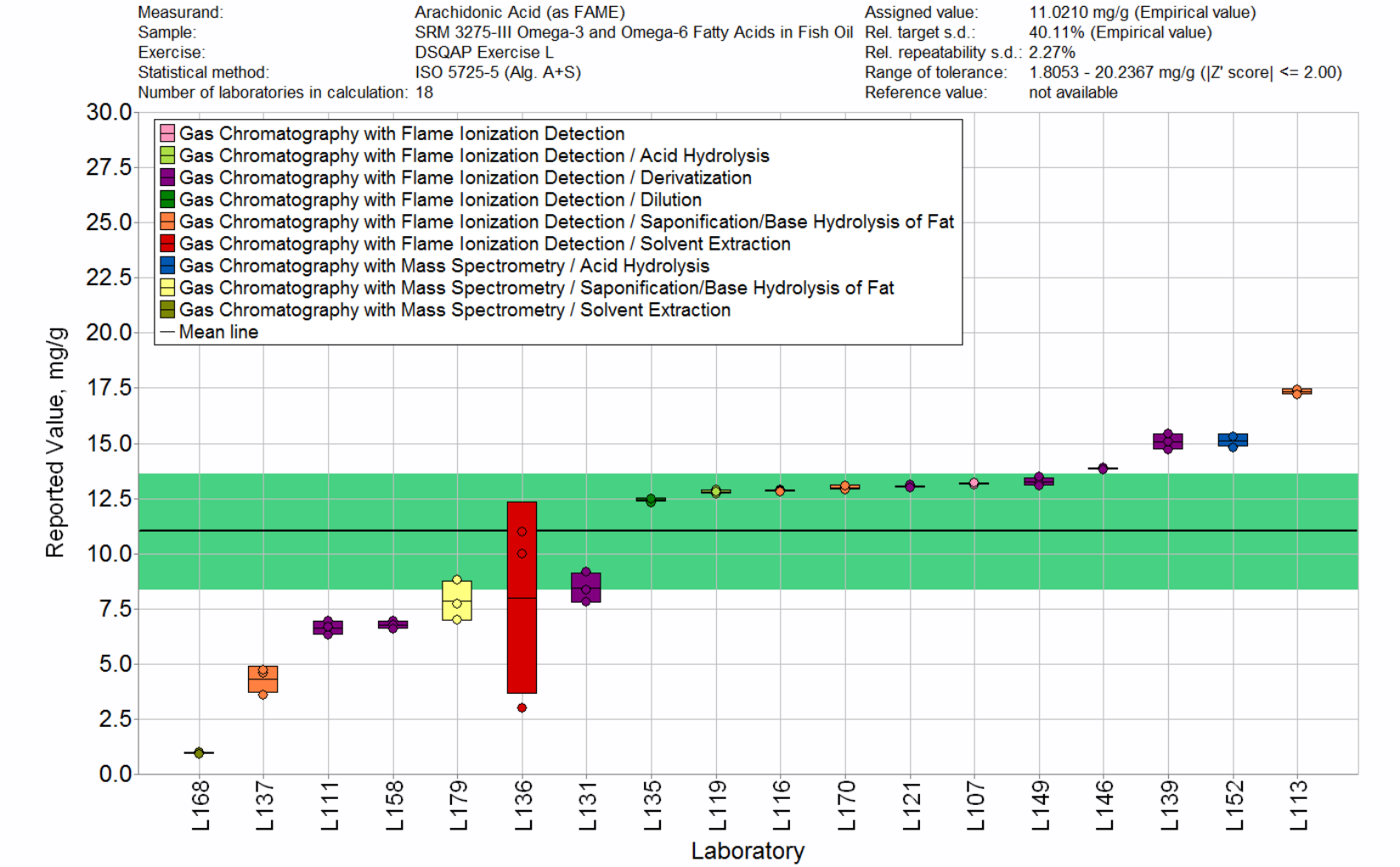

Figure 26. Arachidonic acid in SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. No NIST-determined value is available for this sample.

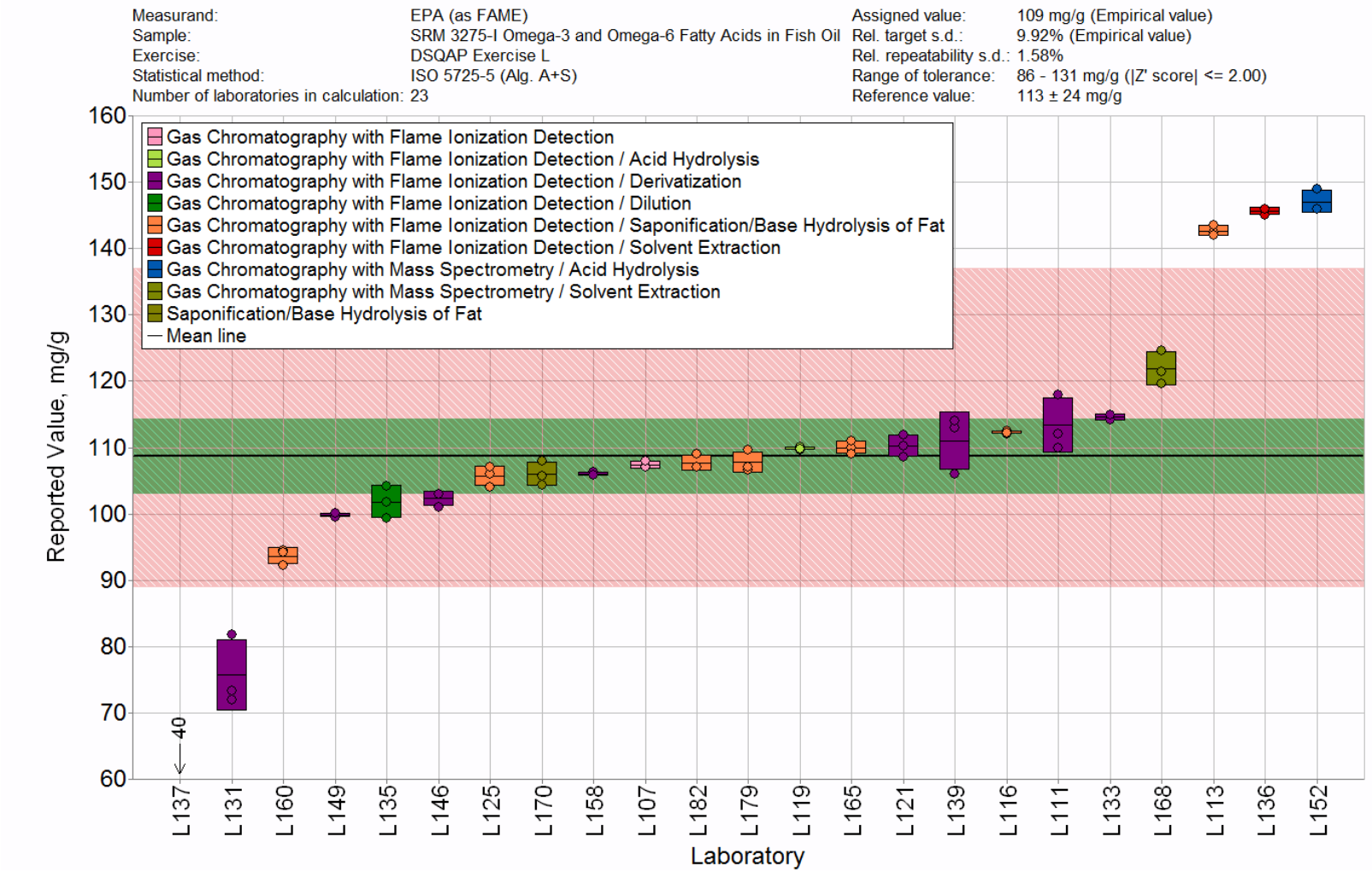

Figure 27. EPA in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST certified value bounded by twice its uncertainty (U_{95}).

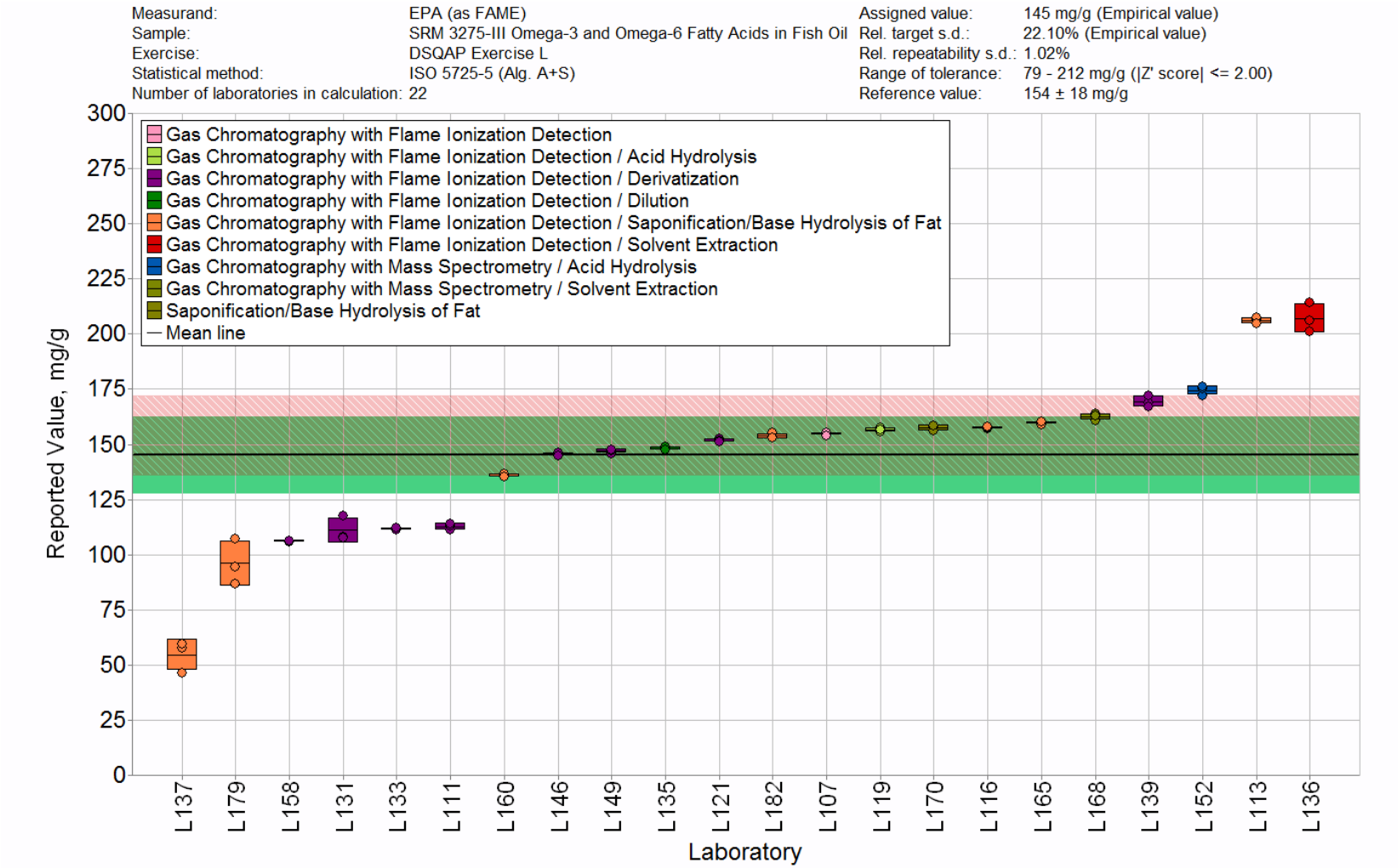

Figure 28. EPA in SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

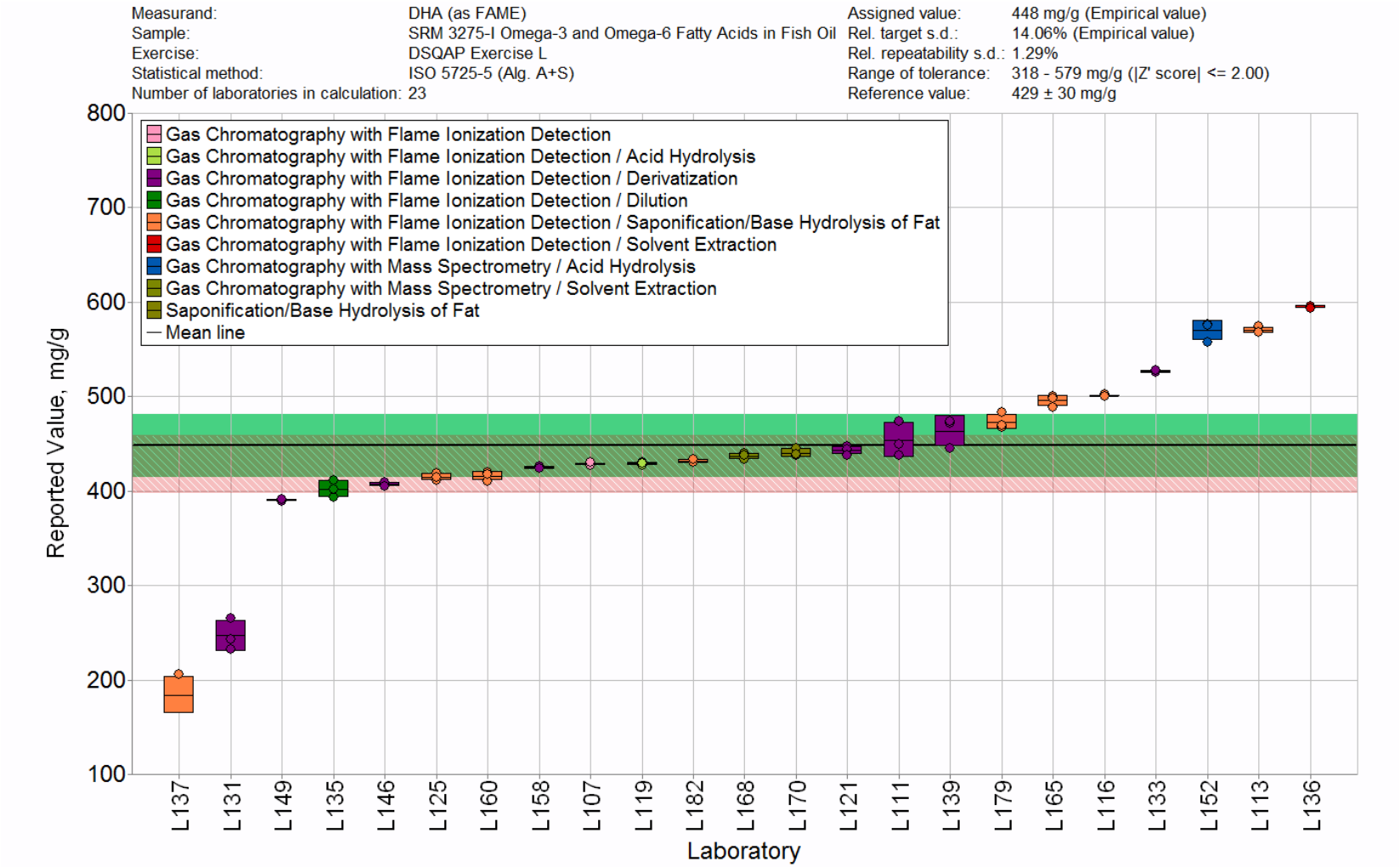

Figure 29. DHA in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

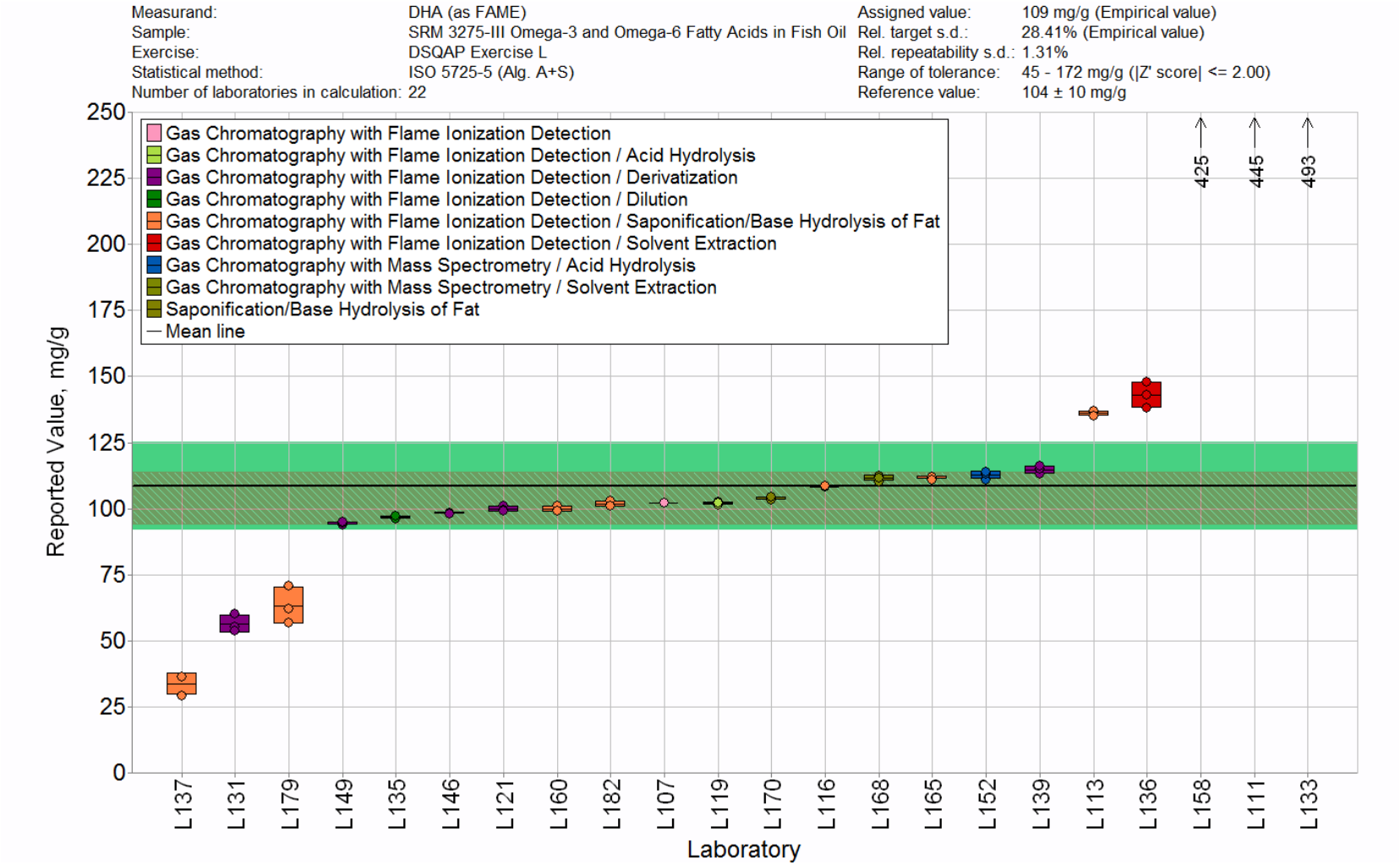

Figure 30. DHA in SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

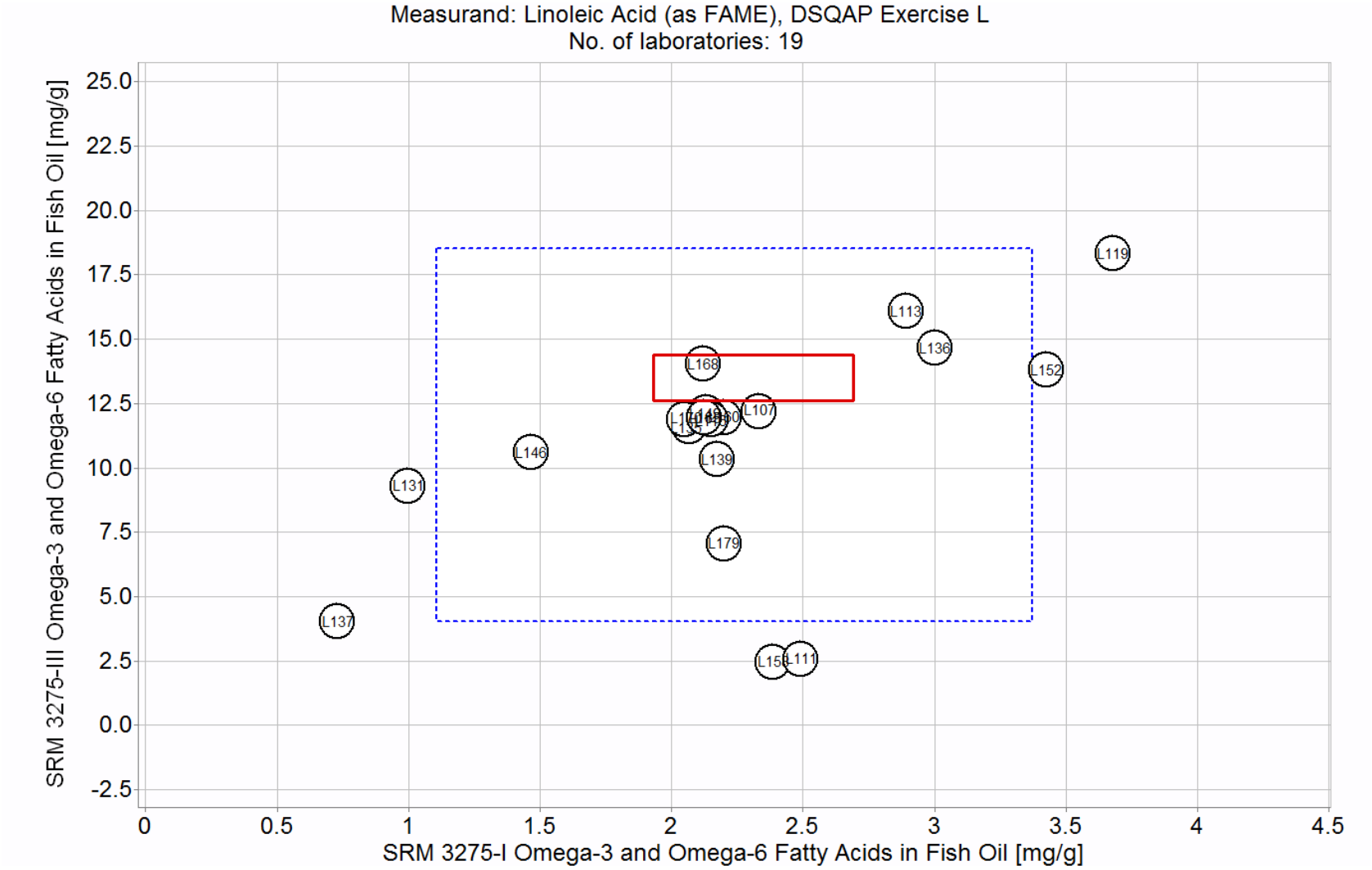

Figure 31. Linoleic acid in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil and SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). In this view, the individual laboratory results for one sample (fish oil 1) are compared to the results for a second sample (fish oil 2). The solid red box represents the target zone for the two samples, fish oil 1 (x-axis) and fish oil 2 (y-axis). The dotted blue box represents the consensus zone for fish oil 1 (x-axis) and fish oil 2 (y-axis).

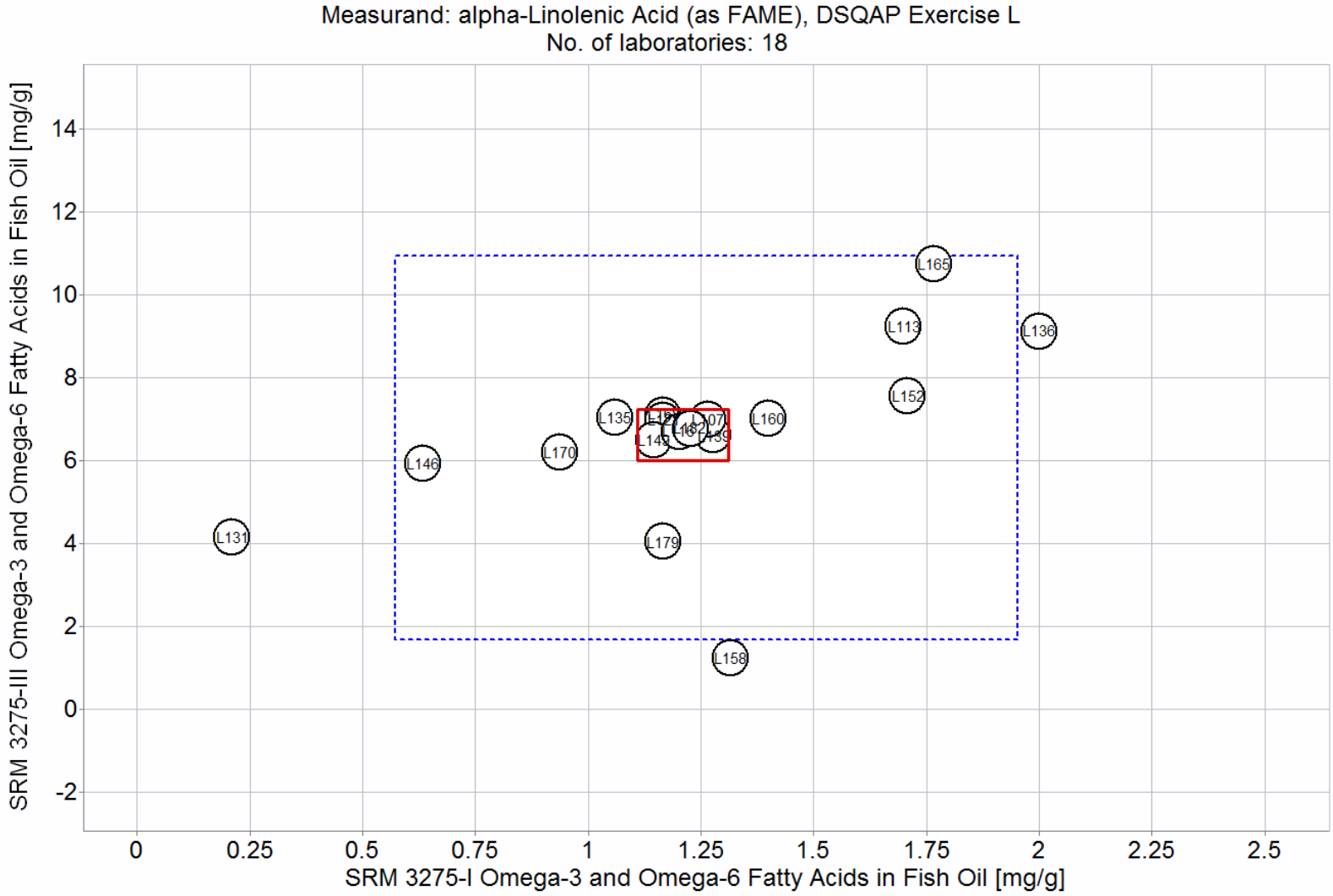

Figure 32. α-Linolenic acid in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil and SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). In this view, the individual laboratory results for one sample (fish oil 1) are compared to the results for a second sample (fish oil 2). The solid red box represents the target zone for the two samples, fish oil 1 (x-axis) and fish oil 2 (y-axis). The dotted blue box represents the consensus zone for fish oil 1 (x-axis) and fish oil 2 (y-axis).

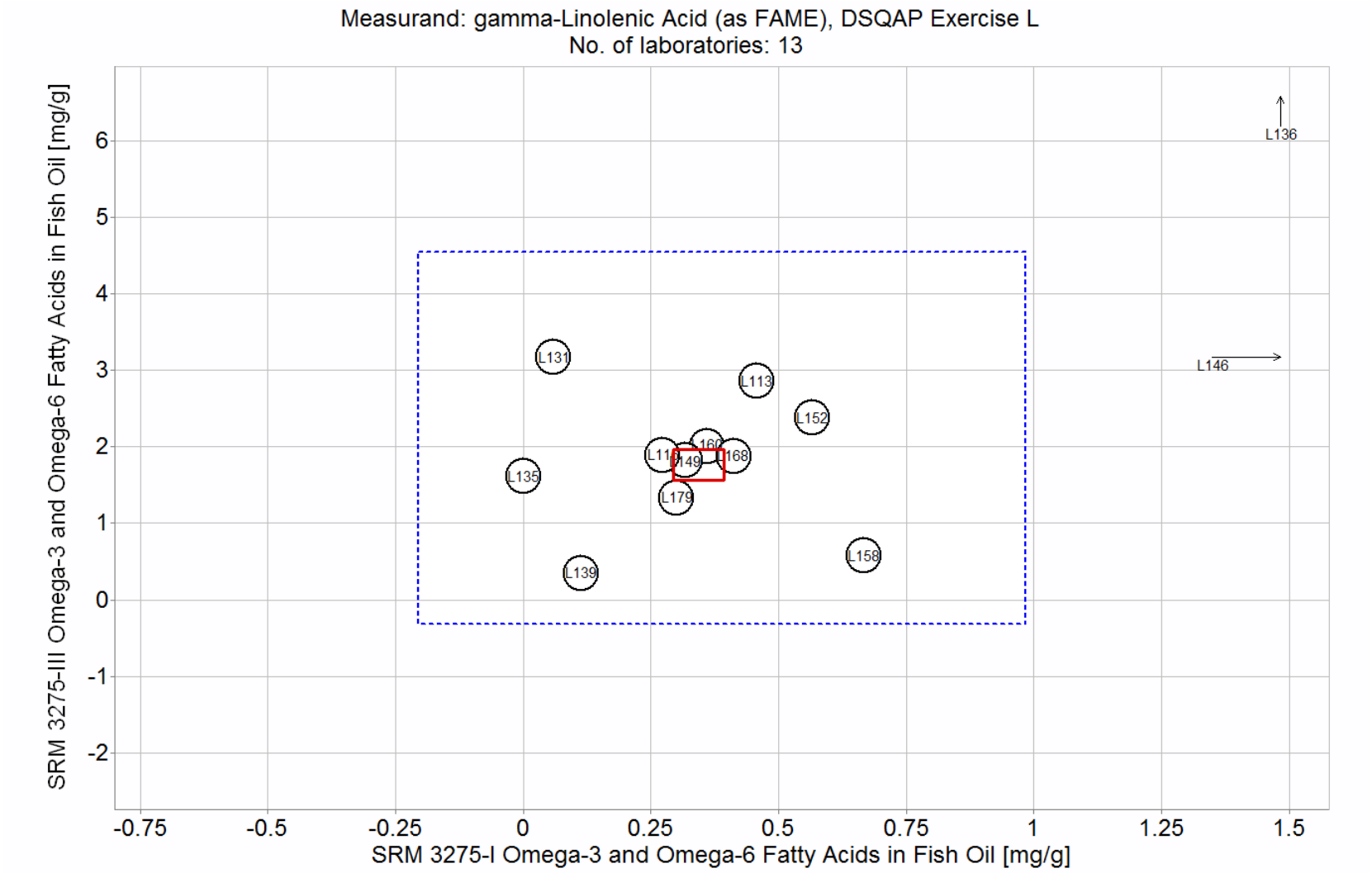

Figure 33. γ-Linolenic acid in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil and SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). In this view, the individual laboratory results for one sample (fish oil 1) are compared to the results for a second sample (fish oil 2). The solid red box represents the target zone for the two samples, fish oil 1 (x-axis) and fish oil 2 (y-axis). The dotted blue box represents the consensus zone for fish oil 1 (x-axis) and fish oil 2 (y-axis).

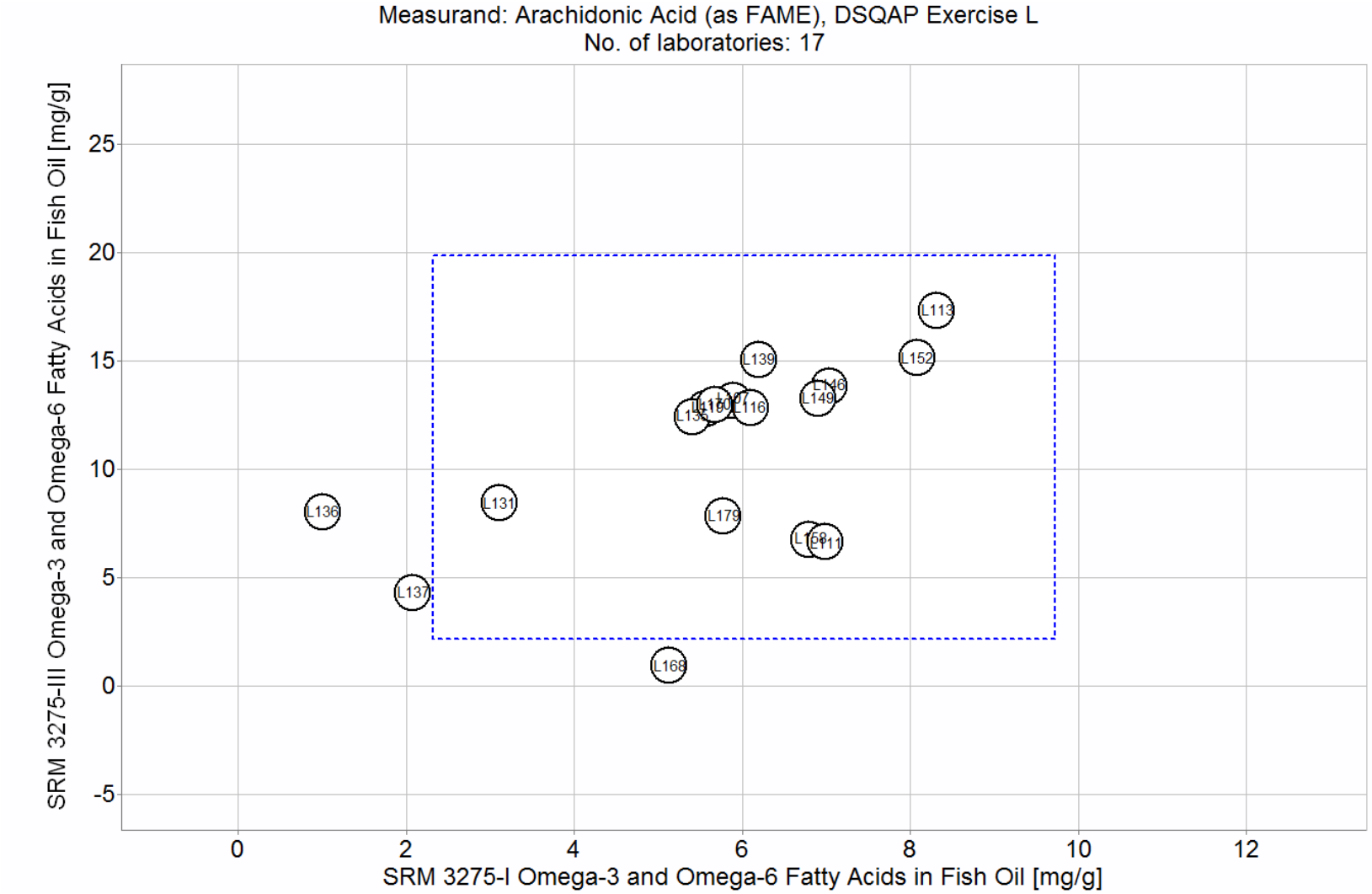

Figure 34. Arachidonic acid in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil and SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). In this view, the individual laboratory results for one sample (fish oil 1) are compared to the results for a second sample (fish oil 2). The dotted blue box represents the consensus zone for fish oil 1 (x-axis) and fish oil 2 (y-axis).

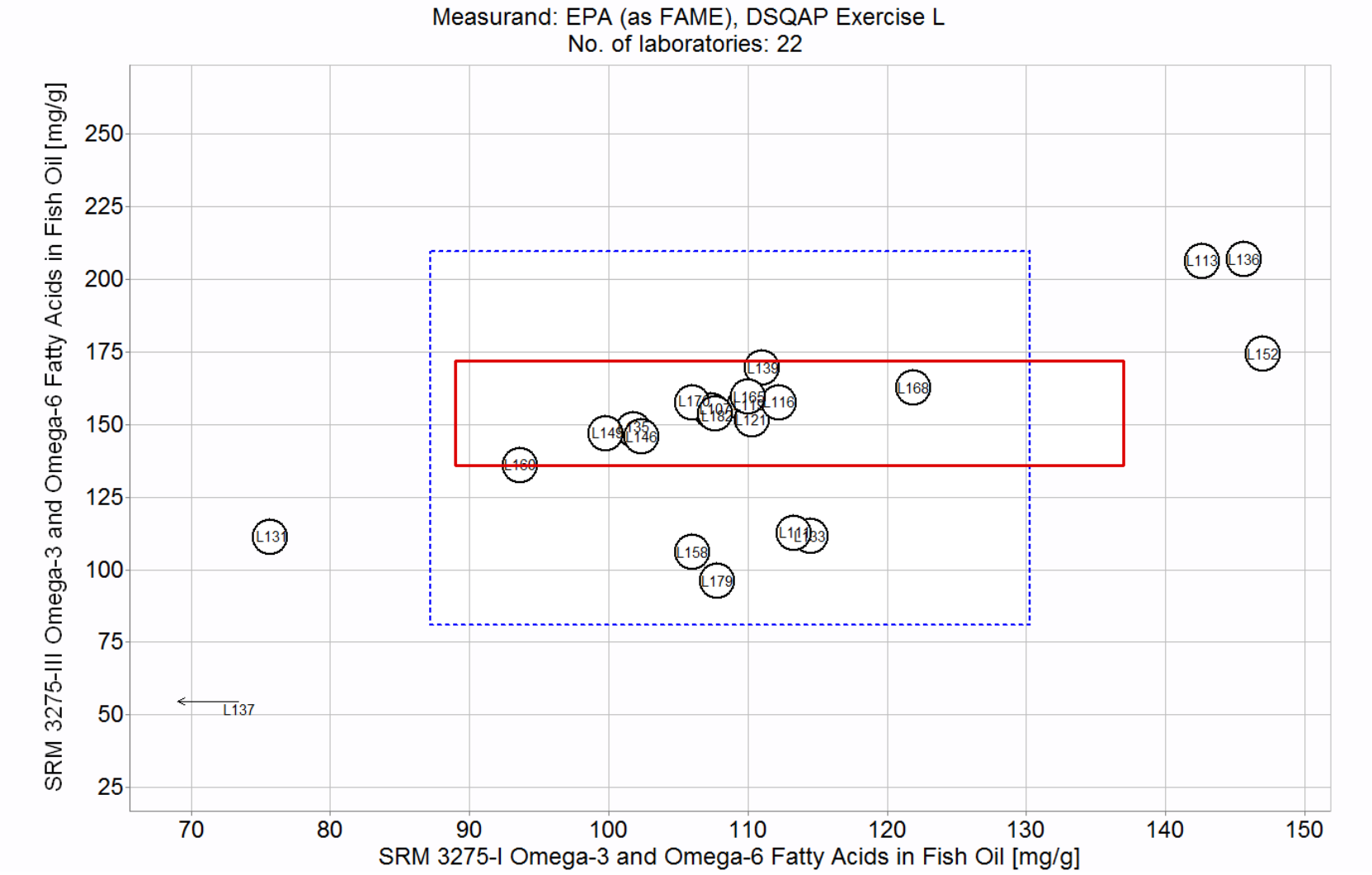

Figure 35. EPA in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil and SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). In this view, the individual laboratory results for one sample (fish oil 1) are compared to the results for a second sample (fish oil 2). The solid red box represents the target zone for the two samples, fish oil 1 (x-axis) and fish oil 2 (y-axis). The dotted blue box represents the consensus zone for fish oil 1 (x-axis) and fish oil 2 (y-axis).

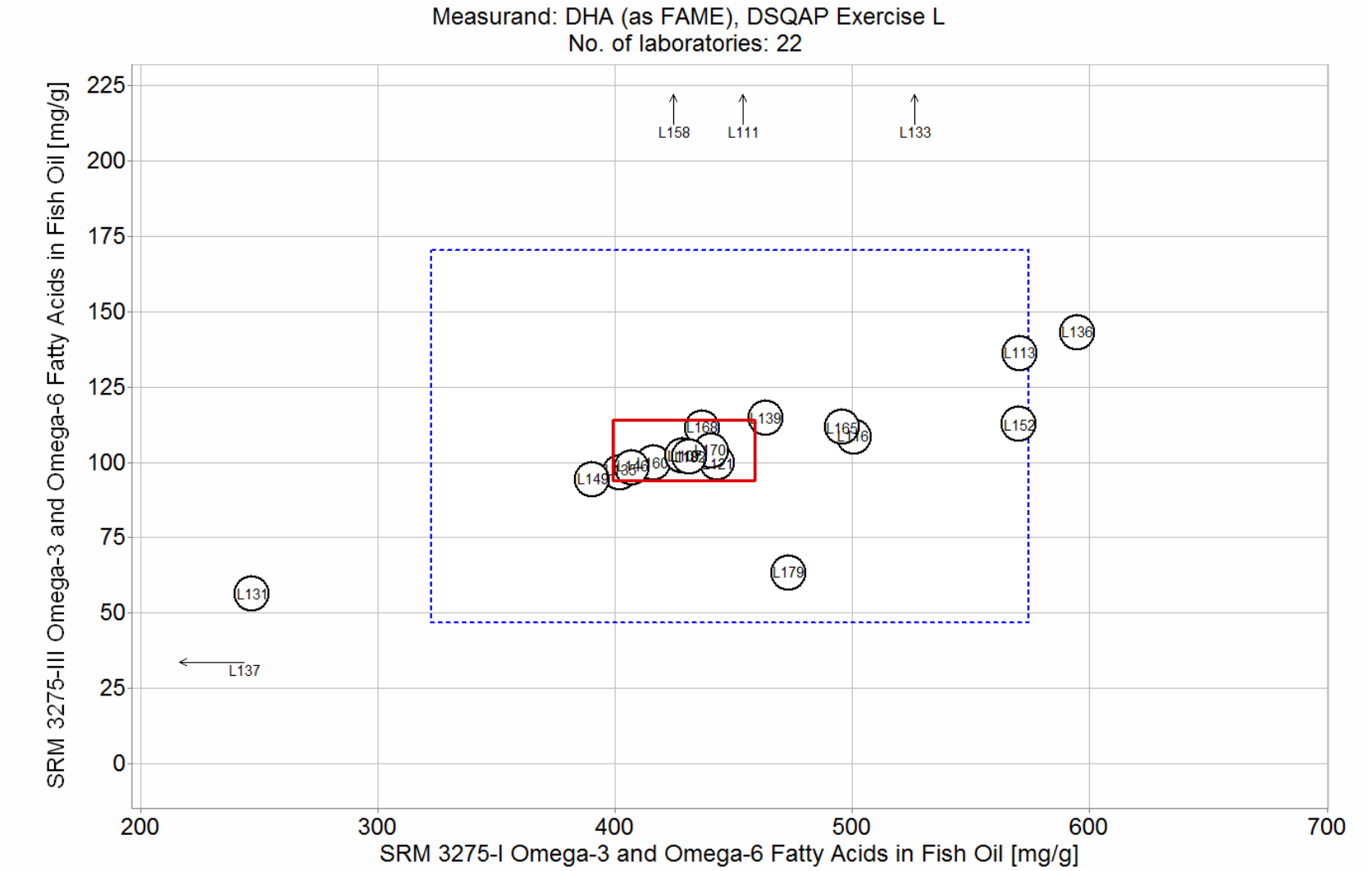

Figure 36. DHA in SRM 3275-I Omega-3 and Omega-6 Fatty Acids in Fish Oil and SRM 3275-III Omega-3 and Omega-6 Fatty Acids in Fish Oil (sample/sample comparison view). In this view, the individual laboratory results for one sample (fish oil 1) are compared to the results for a second sample (fish oil 2). The solid red box represents the target zone for the two samples, fish oil 1 (x-axis) and fish oil 2 (y-axis). The dotted blue box represents the consensus zone for fish oil 1 (x-axis) and fish oil 2 (y-axis).

CHLOROGENIC ACID, FLAVONOIDS, AND NAPHTHODIANTHRONES IN ST. JOHN'S WORT DIETARY SUPPLEMENTS

Study Overview

In this study, participants were provided with two NIST SRMs, SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract. Participants were asked to use in-house analytical methods to determine the mass fractions of chlorogenic acid, rutin, hyperoside, isoquercitrin, quercitrin, quercetin, amentoflavone, pseudohypericin, and hypericin in each of the matrices and report values on an as-received basis.

Sample Information

St. John's Wort Aerial Parts. Participants were provided with three packets containing approximately 3.3 g of dried St. John's Wort aerial parts. The dried leaves were ground, homogenized, and packaged inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and use a sample size of at least 1.0 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. The target values for chlorogenic acid, rutin, hyperoside, quercitrin, pseudohypericin, and hypericin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts were determined at NIST using liquid chromatography with absorbance and fluorescence detection following Soxhlet extraction. Target values for amentoflavone, isoquercitrin, and quercetin have not been established in this material. The NIST-determined values and uncertainties for chlorogenic acid, rutin, hyperoside, quercitrin, pseudohypericin, and hypericin are provided in the table below, on an as-received basis.

Analyte	NIST-Determined Mass Fraction in SRM 3262 (mg/g) (as-received basis)
Chlorogenic acid	0.154 ± 0.007
Rutin	5.05 ± 0.11
Hyperoside	5.02 ± 0.11
Quercitrin	0.984 ± 0.030
Pseudohypericin	0.711 ± 0.020
Hypericin	0.515 ± 0.018

St. John's Wort Methanol Extract. Participants were provided with three packets containing approximately 1.6 g of St. John's Wort methanol extract. The extract was ground, homogenized, and packaged inside 4 mil polyethylene bags, which were then sealed inside nitrogen-flushed aluminized plastic bags along with two packets of silica gel. Before use, participants were instructed to thoroughly mix the contents of each packet and use a sample size of at least 0.1 g. Participants were asked to store the material at controlled room temperature, 10 °C to 30 °C, and to report a single value from each packet provided. Approximate analyte levels were not reported to participants prior to the study. The reference values for chlorogenic acid, rutin, hyperoside,

isoquercitrin, quercitrin, pseudohypericin, and hypericin in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract were determined at NIST using liquid chromatography with absorbance and fluorescence detection following Soxhlet extraction. Target values for amentoflavone and quercetin have not been established in this material. The NIST-determined values and uncertainties for chlorogenic acid, rutin, hyperoside, isoquercitrin, quercitrin, pseudohypericin, and hypericin are provided in the table below, both on a dry-mass basis and on an as-received basis accounting for moisture of the material (0.92 %).

	Reference Mass Fraction in SRM 3264 (mg/g)	
Analyte	(dry-mass basis)	(as-received basis)
Chlorogenic acid	1.050 ± 0.059	1.040 ± 0.058
Rutin	34.3 ± 1.7	34.0 ± 1.7
Hyperoside	17.66 ± 0.88	17.50 ± 0.87
Isoquercitrin	9.47 ± 0.46	9.38 ± 0.46
Quercitrin	3.23 ± 0.16	3.20 ± 0.16
Pseudohypericin	0.809 ± 0.031	0.802 ± 0.031
Hypericin	0.439 ± 0.017	0.435 ± 0.017

Study Results

- Thirty-nine laboratories enrolled in this exercise and received samples. Seventeen laboratories reported data for at least one analyte in the St. John's wort samples (44 % participation).
- The consensus means for rutin in the St. John's wort extract and quercitrin in the St. John's wort aerial parts were within the target ranges with acceptable between-laboratory variability (14 % and 20 % RSD, respectively).
- The consensus means were above the target ranges for chlorogenic acid and hypericin in both samples, as well as for hyperoside, pseudohypericin, and quercitrin in the St. John's wort extract.
  - Observed between-laboratory variability was excellent for chlorogenic acid, hyperoside, and quercitrin in the St. John's wort extract (6 % to 14 % RSD).
  - Between-laboratory variability was extremely high for chlorogenic acid in the St. John's wort aerial parts, for pseudohypericin in St. John's wort extract, and hypericin in both matrices (55 % to 97 % RSD).
- The consensus means were below the target ranges for rutin, hyperoside, and pseudohypericin, in the St. John's wort aerial parts, and for isoquercitrin in the St. John's wort extract.
  - Observed between-laboratory variability was excellent for isoquercitrin in the St. John's wort extract (10 % RSD).
  - Between-laboratory variability was acceptable for rutin and hyperoside in the St. John's wort aerial parts (23 % to 28 % RSD).
  - Between-laboratory variability was extremely high for pseudohypericin in St. John's wort aerial parts (85 % RSD).

- A majority of the laboratories reported using solvent extraction as the sample preparation method (88 %). One laboratory reported using open beaker digestion a sample preparation technique (6 %), and one laboratory reported that no sample preparation was used (6 %).
- A majority of the laboratories reported using LC-absorbance as the analytical approach (82 %). One laboratory reported using UV-VIS (6 %) as their instrumental method, and one laboratory reported using HPLC (6 %).

Technical Recommendations

The following recommendations are based on results obtained from the participants in this study.

- With a small number of laboratories reporting data for these compounds, and a majority reporting use of the same or very similar methods, drawing extensive technical conclusions is difficult.
- No methods presented as significantly better or worse than any other. No systematic biases were noted.
- Some laboratories using LC-absorbance may be experiencing a co-elution that would cause a high bias in the results. The problem can likely be corrected by alteration of the chromatographic conditions. The following recommendations can help identify and avoid potential coelutions.
  - A chromatographic method with alternate selectivity (different retention order) can be used as a check for each new sample type that is run. Ideally, the retention of coeluting compounds would also be affected and the results from the two chromatographic systems would be different. Two different responses would indicate a possible bias in one approach.
  - A different detector can be used in series with an absorbance detector (as confirmation), such as a fluorescence detector or mass spectrometer. If a coeluting compound is present, the response from these detectors would be different than the response from the absorbance detector. Two different responses would indicate a possible bias in one approach.
  - Considerations of potential interferences can assist in troubleshooting. Understanding the matrix that is being tested and possible coeluting compounds can be evaluated before a sample is analyzed for additional confidence in the result.
- Low results for some compounds (such as rutin, isoquercitrin, and hyperoside) may be the result of an incomplete extraction, or only partial hydrolysis of glycosides.
- Calibration materials had a lower purity than expected. It is important to critically evaluate the purity of standards.

Table 18. Individualized data summary table (NIST) for chlorogenic acid, flavonoids, and naphthodianthrones in St. John's wort dietary supplements.

National Institute of Standards & Technology

Exercise L - October 2015 - Botanical Analytes

	Lab Code:	NIST	1. Your Results				2. Community Results			3. Target	
Analyte	Sample	Units	x_i	s_i	Z'_{comm}	Z_{NIST}	N	x*	s*	x_{NIST}	U_{95}
Chlorogenic Acid	SJW Aerial Parts	mg/g	0.154	0.015		0.00	8	0.186	0.130	0.154	0.015
Chlorogenic Acid	SJW Extract	mg/g	1.04	0.12		0.00	9	1.26	0.17	1.04	0.12
Rutin	SJW Aerial Parts	mg/g	5.05	0.22		0.00	17	3.57	0.84	5.05	0.22
Rutin	SJW Extract	mg/g	34.0	3.4		0.00	17	30.9	4.5	34.0	3.4
Hyperoside	SJW Aerial Parts	mg/g	5.02	0.22		0.00	11	3.48	0.98	5.02	0.22
Hyperoside	SJW Extract	mg/g	17.5	1.7		0.00	11	20.3	1.2	17.5	1.7
Isoquercitrin	SJW Aerial Parts	mg/g					8	1.44	0.37		
Isoquercitrin	SJW Extract	mg/g	9.38	0.91		0.00	8	7.58	0.72	9.38	0.91
Quercitrin	SJW Aerial Parts	mg/g	0.984	0.060		0.00	10	0.952	0.191	0.984	0.060
Quercitrin	SJW Extract	mg/g	3.20	0.32		0.00	10	3.94	0.43	3.20	0.32
Quercetin	SJW Aerial Parts	mg/g					16	2.01	0.32		
Quercetin	SJW Extract	mg/g					16	6.44	0.80		
Amentoflavone	SJW Aerial Parts	mg/g					2	0.0370	0.0040		
Amentoflavone	SJW Extract	mg/g					2	0.0980	0.0040		
Pseudohypericin	SJW Aerial Parts	mg/g	0.711	0.040		0.00	6	0.605	0.514	0.711	0.040
Pseudohypericin	SJW Extract	mg/g	0.802	0.061		0.00	6	1.310	0.726	0.802	0.061
Hypericin	SJW Aerial Parts	mg/g	0.515	0.036		0.00	7	0.781	0.676	0.515	0.036
Hypericin	SJW Extract	mg/g	0.435	0.034		0.00	8	1.609	1.560	0.435	0.034

x_i Mean of reported values

s_i Standard deviation of reported values

Z'_{comm} Z'-score with respect to community consensus

Z_{NIST} Z-score with respect to NIST value

N Number of quantitative values reported

x* Robust mean of reported values

s* Robust standard deviation

x_{NIST} NIST-assessed value

U_{95} ±95% confidence interval about the assessed value or standard deviation (s_{NIST})

Table 19. Data summary table for chlorogenic acid in St. John's wort dietary supplements.

		Chlorogenic Acid									
		SRM 3262 St. John's Wort Aerial Parts (mg/g)					SRM 3264 St. John's Wort Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.154	0.015				1.04	0.12
	L101										
	L102	0.760	0.820	0.630	0.737	0.097	3.47	3.38	3.54	3.46	0.08
	L103	< 0.100	< 0.100	< 0.100			1.04	1.10	1.09	1.08	0.03
	L104										
	L105										
	L107										
	L110	0.245	0.246	0.246	0.246	0.001	1.26	1.33	1.31	1.30	0.04
	L111	0.225	0.224	0.223	0.224	0.001	1.24	1.31	1.31	1.29	0.04
	L112										
	L113										
	L118										
	L120										
	L122										
	L125	0.188	0.191	0.207	0.195	0.010	1.30	1.29	1.29	1.29	0.01
	L126										
	L128	0.140	0.110	0.130	0.127	0.015	1.38	1.36	1.38	1.37	0.01
	L130										
	L131										
	L133										
	L137										
	L138	0.080	0.080	0.070	0.077	0.006	1.24	1.25	1.26	1.25	0.01
	L139										
	L141										
	L144	0.010	0.010		0.010	0.000	0.80	0.70		0.75	0.07
	L150										
	L151										
	L153										
	L155										
	L157										
	L159										
	L160										
	L163										
	L164										
	L165	0.230	0.230	0.230	0.230	0.000	1.24	1.24	1.25	1.24	0.01
	L166										
	L170										
	L172										
	L177										
	L179										
Community Results		Consensus Mean			0.186		Consensus Mean			1.26	
		Consensus Standard Deviation			0.130		Consensus Standard Deviation			0.17	
		Maximum			0.737		Maximum			3.46	
		Minimum			0.010		Minimum			0.75	
		N			8		N			9	

Table 20. Data summary table for rutin in St. John's wort dietary supplements.

		Rutin									
		SRM 3262 St. John's Wort Aerial Parts (mg/g)					SRM 3264 St. John's Wort Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				5.05	0.22				34.0	3.4
	L101										
	L102	5.68	5.98	6.35	6.00	0.34	8.3	9.3	9.7	9.1	0.7
	L103										
	L104										
	L105										
	L107										
	L110	5.46	5.53	5.43	5.47	0.05	32.3	35.2	33.4	33.6	1.5
	L111	3.15	3.05	2.87	3.02	0.14	29.1	29.2	29.5	29.3	0.2
	L112										
	L113										
	L118										
	L120	4.21	3.70	3.69	3.87	0.30	33.1	33.6	33.0	33.2	0.3
	L122										
	L126	3.60	3.32	3.94	3.62	0.31	34.2	34.0	33.9	34.0	0.2
	L128										
	L130										
	L131										
	L133	2.31	2.20	2.11	2.21	0.10	29.1	27.9	29.4	28.8	0.8
	L137	50.25	46.97	48.28	48.50	1.65	249.9	241.5	253.5	248.3	6.2
	L138	3.23	3.25	3.14	3.21	0.06	31.1	30.9	31.2	31.1	0.1
	L139	3.25	3.82	3.31	3.46	0.31	14.9	15.5	14.5	15.0	0.5
	L140	3.75	4.26	3.40	3.80	0.43	33.9	33.3	33.7	33.6	0.3
	L141	3.27	3.51	3.67	3.48	0.20	33.8	33.3	32.4	33.2	0.7
	L144	3.80	3.50		3.65	0.21	33.4	33.5		33.5	0.1
	L150										
	L151	3.32	3.64	3.75	3.57	0.22	34.6	34.8	34.8	34.8	0.1
	L153										
	L155										
	L157	2.76	3.62	3.43	3.27	0.45	31.2	31.9	31.0	31.3	0.5
	L159										
	L160										
	L163										
	L164										
	L165	1.60	1.70	1.68	1.66	0.05	15.7	16.0	15.9	15.9	0.2
	L166										
	L170										
	L171	3.44	3.45	3.52	3.47	0.04	29.7	30.6	29.2	29.8	0.7
	L172										
	L177										
	L179	3.25	3.21	3.24	3.23	0.02	29.0	29.1	29.0	29.0	0.1
Community Results		Consensus Mean			3.57		Consensus Mean			30.9	
		Consensus Standard Deviation			0.84		Consensus Standard Deviation			4.5	
		Maximum			48.50		Maximum			248.3	
		Minimum			1.66		Minimum			9.1	
		N			17		N			17	

Table 21. Data summary table for hyperoside in St. John's wort dietary supplements.

		Rutin									
		SRM 3262 St. John's Wort Aerial Parts (mg/g)					SRM 3264 St. John's Wort Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				5.05	0.22				34.0	3.4
	L101										
	L102	5.68	5.98	6.35	6.00	0.34	8.3	9.3	9.7	9.1	0.7
	L103										
	L104										
	L105										
	L107										
	L110	5.46	5.53	5.43	5.47	0.05	32.3	35.2	33.4	33.6	1.5
	L111	3.15	3.05	2.87	3.02	0.14	29.1	29.2	29.5	29.3	0.2
	L112										
	L113										
	L118										
	L120	4.21	3.70	3.69	3.87	0.30	33.1	33.6	33.0	33.2	0.3
	L122										
	L126	3.60	3.32	3.94	3.62	0.31	34.2	34.0	33.9	34.0	0.2
	L128										
	L130										
	L131										
	L133	2.31	2.20	2.11	2.21	0.10	29.1	27.9	29.4	28.8	0.8
	L137	50.25	46.97	48.28	48.50	1.65	249.9	241.5	253.5	248.3	6.2
	L138	3.23	3.25	3.14	3.21	0.06	31.1	30.9	31.2	31.1	0.1
	L139	3.25	3.82	3.31	3.46	0.31	14.9	15.5	14.5	15.0	0.5
	L140	3.75	4.26	3.40	3.80	0.43	33.9	33.3	33.7	33.6	0.3
	L141	3.27	3.51	3.67	3.48	0.20	33.8	33.3	32.4	33.2	0.7
	L144	3.80	3.50		3.65	0.21	33.4	33.5		33.5	0.1
	L150										
	L151	3.32	3.64	3.75	3.57	0.22	34.6	34.8	34.8	34.8	0.1
	L153										
	L155										
	L157	2.76	3.62	3.43	3.27	0.45	31.2	31.9	31.0	31.3	0.5
	L159										
	L160										
	L163										
	L164										
	L165	1.60	1.70	1.68	1.66	0.05	15.7	16.0	15.9	15.9	0.2
	L166										
	L170										
	L171	3.44	3.45	3.52	3.47	0.04	29.7	30.6	29.2	29.8	0.7
	L172										
	L177										
	L179	3.25	3.21	3.24	3.23	0.02	29.0	29.1	29.0	29.0	0.1
Community Results		Consensus Mean			3.57		Consensus Mean			30.9	
		Consensus Standard Deviation			0.84		Consensus Standard Deviation			4.5	
		Maximum			48.50		Maximum			248.3	
		Minimum			1.66		Minimum			9.1	
		N			17		N			17	

Table 22. Data summary table for isoquercitrin in St. John's wort dietary supplements.

		Isoquercitrin									
		SRM 3262 St. John's Wort Aerial Parts (mg/g)					SRM 3264 St. John's Wort Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST									9.38	0.91
	L101										
	L103										
	L104										
	L105										
	L107										
	L110	2.43	2.45	2.41	2.43	0.02	7.85	8.56	8.04	8.15	0.37
	L112										
	L113										
	L120	1.58	1.40	1.34	1.44	0.12	7.12	7.59	7.43	7.38	0.24
	L122										
	L126	1.33	1.24	1.06	1.21	0.13	6.43	6.49	6.36	6.42	0.06
	L128										
	L130										
	L131										
	L137										
	L138	1.83	1.85	1.77	1.82	0.04	12.09	12.03	12.15	12.09	0.06
	L139										
	L140	1.47	1.39	1.52	1.46	0.07	7.49	7.61	7.99	7.70	0.26
	L141	1.17	1.31	1.39	1.29	0.11	7.73	7.46	7.43	7.54	0.16
	L151	0.99	1.00	1.01	1.00	0.01	7.33	7.34	7.42	7.36	0.05
	L153										
	L155										
	L157	1.02	1.44	1.37	1.28	0.22	7.34	7.44	7.27	7.35	0.09
	L159										
	L163										
	L164										
	L166										
	L170										
	L172										
	L177										
	L179										
Community Results		Consensus Mean			1.44		Consensus Mean			7.58	
		Consensus Standard Deviation			0.37		Consensus Standard Deviation			0.72	
		Maximum			2.43		Maximum			12.09	
		Minimum			1.00		Minimum			6.42	
		N			8		N			8	

Table 23. Data summary table for quercitrin in St. John's wort dietary supplements.

		Quercitrin									
		SRM 3262 St. John's Wort Aerial Parts (mg/g)					SRM 3264 St. John's Wort Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.984	0.060				3.20	0.32
	L101										
	L103										
	L104										
	L105										
	L107										
	L110	1.210	1.220	1.190	1.207	0.015	3.59	3.90	3.70	3.73	0.16
	L112										
	L113										
	L120	1.022	0.951	0.962	0.978	0.038	3.75	3.81	3.84	3.80	0.05
	L122										
	L126	1.056	0.987	0.936	0.993	0.060	3.91	3.88	3.93	3.91	0.03
	L128										
	L130										
	L131										
	L137	0.710	0.687	0.733	0.710	0.023	3.40	3.44	3.33	3.39	0.06
	L138	0.928	0.931	0.906	0.922	0.014	3.63	3.61	3.63	3.62	0.01
	L139										
	L140	0.800	0.740	0.820	0.787	0.042	5.44	5.80	5.66	5.63	0.18
	L141	0.899	0.928	0.952	0.926	0.027	3.99	3.89	3.93	3.94	0.05
	L144	2.100	1.900		2.000	0.141	6.20	6.20		6.20	0.00
	L151	0.837	0.824	0.827	0.829	0.007	3.88	4.03	3.95	3.95	0.08
	L153										
	L155										
	L157	0.823	1.003	0.972	0.933	0.096	3.90	3.89	3.84	3.88	0.03
	L159										
	L163										
	L164										
	L166										
	L170										
	L172										
	L177										
	L179										
Community Results		Consensus Mean			0.952		Consensus Mean			3.94	
		Consensus Standard Deviation			0.191		Consensus Standard Deviation			0.43	
		Maximum			2.000		Maximum			6.20	
		Minimum			0.710		Minimum			3.39	
		N			10		N			10	

Table 24. Data summary table for quercetin in St. John's wort dietary supplements.

		Quercetin									
		SRM 3262 St. John's Wort Aerial Parts (mg/g)					SRM 3264 St. John's Wort Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST										
	L101										
	L102	2.07	2.15	2.25	2.16	0.09	5.96	5.99	6.06	6.00	0.05
	L103	23.90	24.30	24.80	24.33	0.45	98.00	98.60	94.50	97.03	2.21
	L104										
	L105										
	L107										
	L110	2.38	2.38	2.35	2.37	0.02	6.09	6.59	6.27	6.32	0.25
	L111	1.93	1.83	1.78	1.85	0.08	5.43	5.45	5.51	5.46	0.04
	L112										
	L113										
	L118										
	L120	1.80	1.79	1.77	1.78	0.02	6.23	6.35	6.23	6.27	0.07
	L122										
	L126	1.98	1.96	2.00	1.98	0.02	7.47	7.25	7.47	7.40	0.13
	L128										
	L130										
	L131										
	L133	1.54	1.49	1.43	1.49	0.06	6.07	5.73	5.81	5.87	0.18
	L134										
	L137	1.92	1.88	1.94	1.91	0.03	5.63	5.58	5.55	5.59	0.04
	L138	2.14	2.16	2.09	2.13	0.04	6.34	6.27	6.33	6.31	0.04
	L139	2.19	2.30	2.20	2.23	0.06	5.99	6.09	5.85	5.98	0.12
	L140	1.89	1.86	1.82	1.86	0.04	6.63	6.38	6.59	6.53	0.13
	L141	1.77	1.71	1.71	1.73	0.03	6.80	6.70	6.53	6.68	0.14
	L144										
	L150										
	L151	1.97	1.94	1.99	1.97	0.02	7.47	7.54	7.47	7.49	0.04
	L153										
	L155										
	L157	1.54	1.63	1.60	1.59	0.04	5.89	5.96	5.73	5.86	0.12
	L159										
	L160										
	L163										
	L164										
	L165	2.12	2.11	2.10	2.11	0.01	6.08	6.23	6.16	6.16	0.08
	L166										
	L170										
	L171	1.91	1.92	2.00			6.33	6.38	6.37		
	L172										
	L177										
	L179	28.29	28.03	28.23	28.18	0.14	105.01	108.92	103.23	105.72	2.91
Community Results		Consensus Mean			2.01		Consensus Mean			6.44	
		Consensus Standard Deviation			0.32		Consensus Standard Deviation			0.80	
		Maximum			28.18		Maximum			105.72	
		Minimum			1.49		Minimum			5.46	
		N			16		N			16	

Table 25. Data summary table for amentoflavone in St. John's wort dietary supplements.

		Amentoflavone									
		SRM 3262 St. John's Wort Aerial Parts (mg/g)					SRM 3264 St. John's Wort Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST										
	L103										
	L104										
	L105										
	L107										
	L110	0.0410	0.0380	0.0370	0.0387	0.0021	0.0930	0.0990	0.0960	0.0960	0.0030
	L112										
	L120										
	L122										
	L126										
	L128										
	L130										
	L137										
	L138	0.0340	0.0350	0.0340	0.0343	0.0006	0.1050	0.0990	0.0950	0.0997	0.0050
	L139										
	L141										
	L151										
	L153										
	L155										
	L157										
	L159										
	L163										
	L164										
	L166										
	L170										
	L172										
	L177										
	L179										
Community Results		Consensus Mean			0.0370		Consensus Mean			0.0980	
		Consensus Standard Deviation			0.0040		Consensus Standard Deviation			0.0040	
		Maximum			0.0387		Maximum			0.0997	
		Minimum			0.0343		Minimum			0.0960	
		N			2		N			2	

Table 26. Data summary table for pseudohypericin in St. John's wort dietary supplements.

		Pseudohypericin									
		SRM 3262 St. John's Wort Aerial Parts (mg/g)					SRM 3264 St. John's Wort Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.711	0.040				0.802	0.061
	L102	0.360	0.360	0.360	0.360	0.000	1.760	1.770	1.760	1.763	0.006
	L103										
	L104										
	L105										
	L107										
	L110	1.090	1.050	1.090	1.077	0.023	1.230	1.210	1.230	1.223	0.012
	L111	1.080	1.070	1.040	1.063	0.021	1.460	1.480	1.480	1.473	0.012
	L112										
	L120										
	L122										
	L126										
	L128										
	L130										
	L131										
	L133										
	L137	0.875	0.885	0.872	0.877	0.007	2.067	2.250	2.199	2.172	0.094
	L138										
	L139	0.081	0.082	0.084	0.082	0.002	0.810	0.850	0.840	0.833	0.021
	L141										
	L144										
	L151										
	L153										
	L155										
	L157										
	L159										
	L163										
	L164										
	L165	0.134	0.206	0.179	0.173	0.036	0.415	0.360	0.412	0.396	0.031
	L166										
	L170										
	L172										
	L177										
	L179										
Community Results		Consensus Mean			0.605		Consensus Mean			1.310	
		Consensus Standard Deviation			0.514		Consensus Standard Deviation			0.726	
		Maximum			1.077		Maximum			2.172	
		Minimum			0.082		Minimum			0.396	
		N			6		N			6	

Table 27. Data summary table for hypericin in St. John's wort dietary supplements.

		Hypericin									
		SRM 3262 St. John's Wort Aerial Parts (mg/g)					SRM 3264 St. John's Wort Extract (mg/g)				
	Lab	A	B	C	Avg	SD	A	B	C	Avg	SD
Individual Results	NIST				0.515	0.036				0.435	0.034
	L102	1.460	1.290	1.340	1.363	0.087	1.550	1.570	1.560	1.560	0.010
	L103						5.600	4.900	5.500	5.333	0.379
	L104										
	L105										
	L107										
	L110	0.453	0.435	0.454	0.447	0.011	0.390	0.397	0.401	0.396	0.006
	L111	0.607	0.592	0.585	0.595	0.011	0.603	0.676	0.636	0.638	0.037
	L112										
	L118										
	L120										
	L122										
	L124										
	L126										
	L128										
	L130										
	L131										
	L133										
	L134										
	L137	0.767	0.719	0.730	0.739	0.025	1.756	1.859	1.786	1.800	0.053
	L138										
	L139	0.008	0.008	0.009	0.008	0.001	0.170	0.180	0.150	0.167	0.015
	L141										
	L144										
	L150										
	L151										
	L153										
	L155										
	L157										
	L159										
	L163										
	L164										
	L165	0.536	0.528	0.553	0.539	0.013	1.053	1.003	1.080	1.045	0.039
	L166										
	L170	1.810	1.770	1.750	1.777	0.031	3.310	3.330	3.310	3.317	0.012
	L172										
	L177										
	L179										
Community Results		Consensus Mean			0.781		Consensus Mean			1.609	
		Consensus Standard Deviation			0.676		Consensus Standard Deviation			1.560	
		Maximum			1.777		Maximum			5.333	
		Minimum			0.008		Minimum			0.167	
		N			7		N			8	

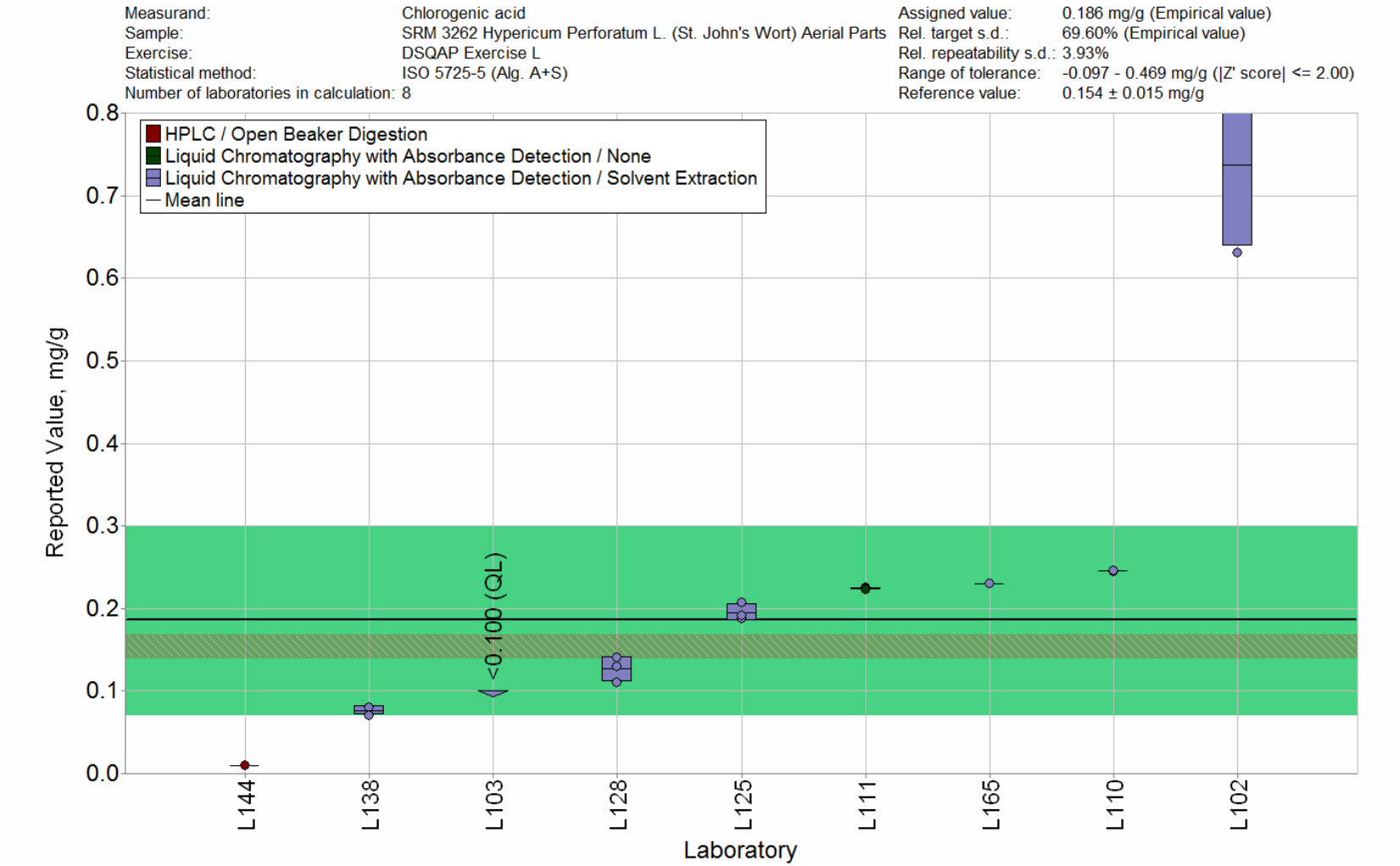

Figure 37. Chlorogenic acid in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

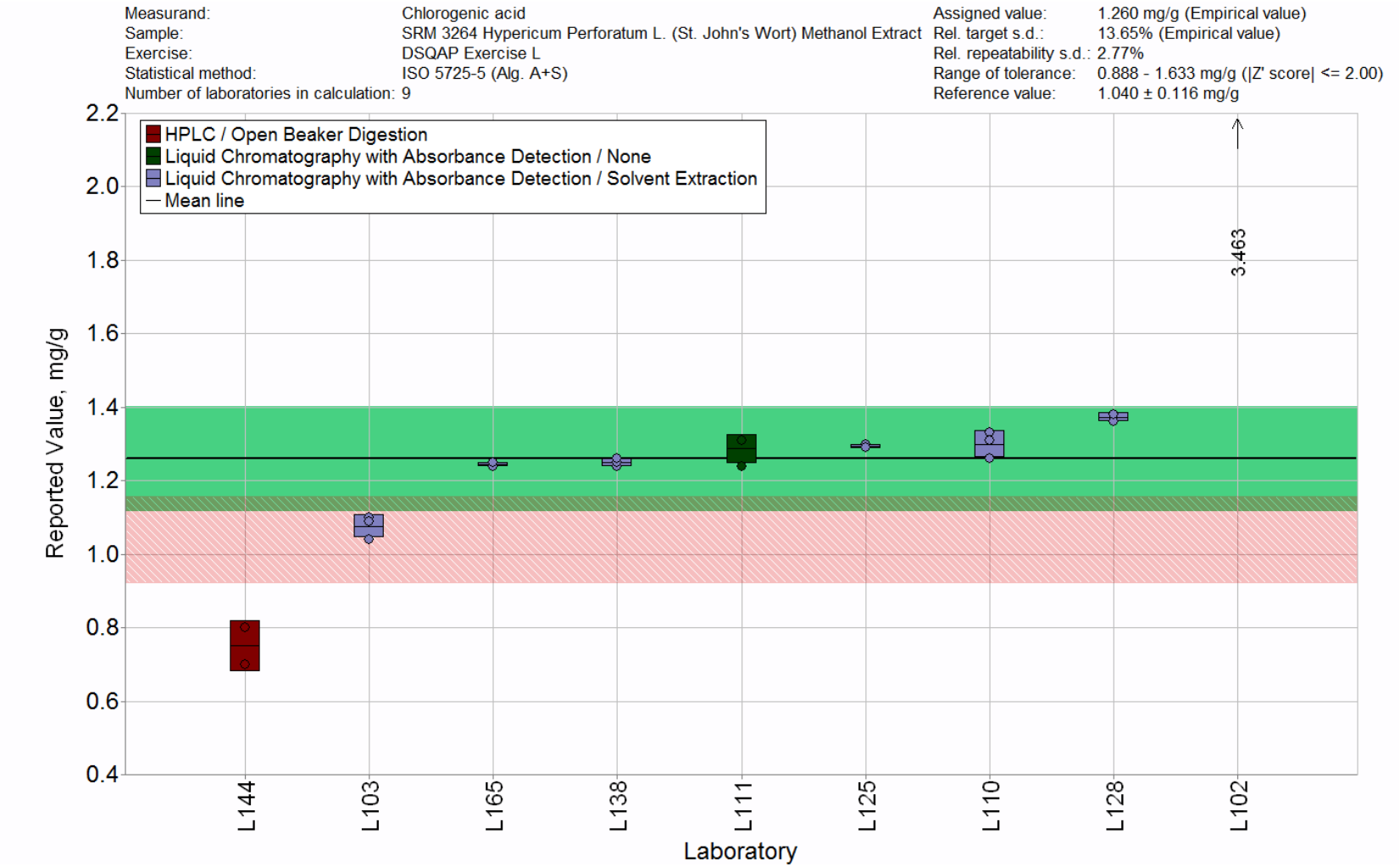

Figure 38. Chlorogenic acid in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

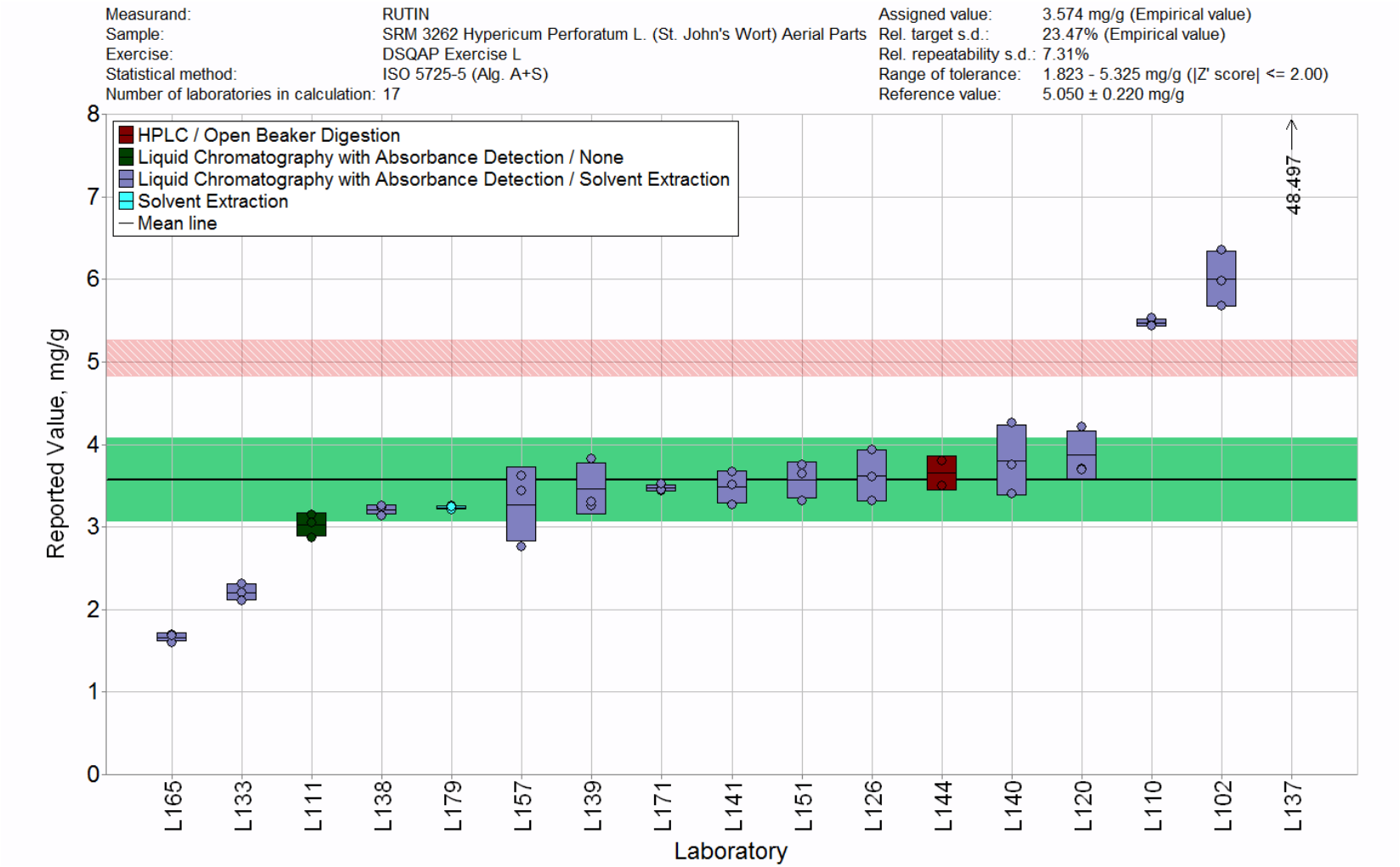

Figure 39. Rutin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

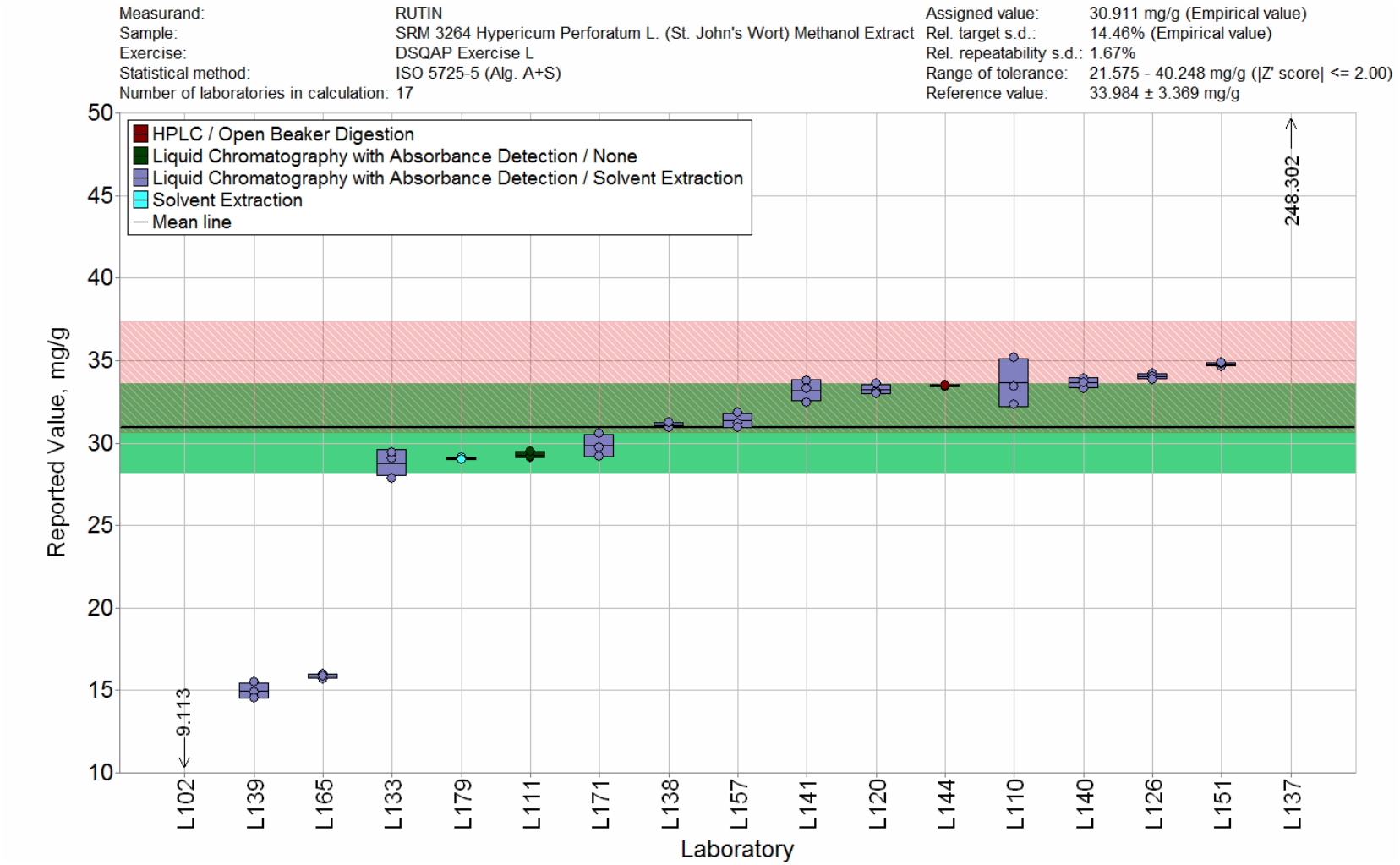

Figure 40. Rutin in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

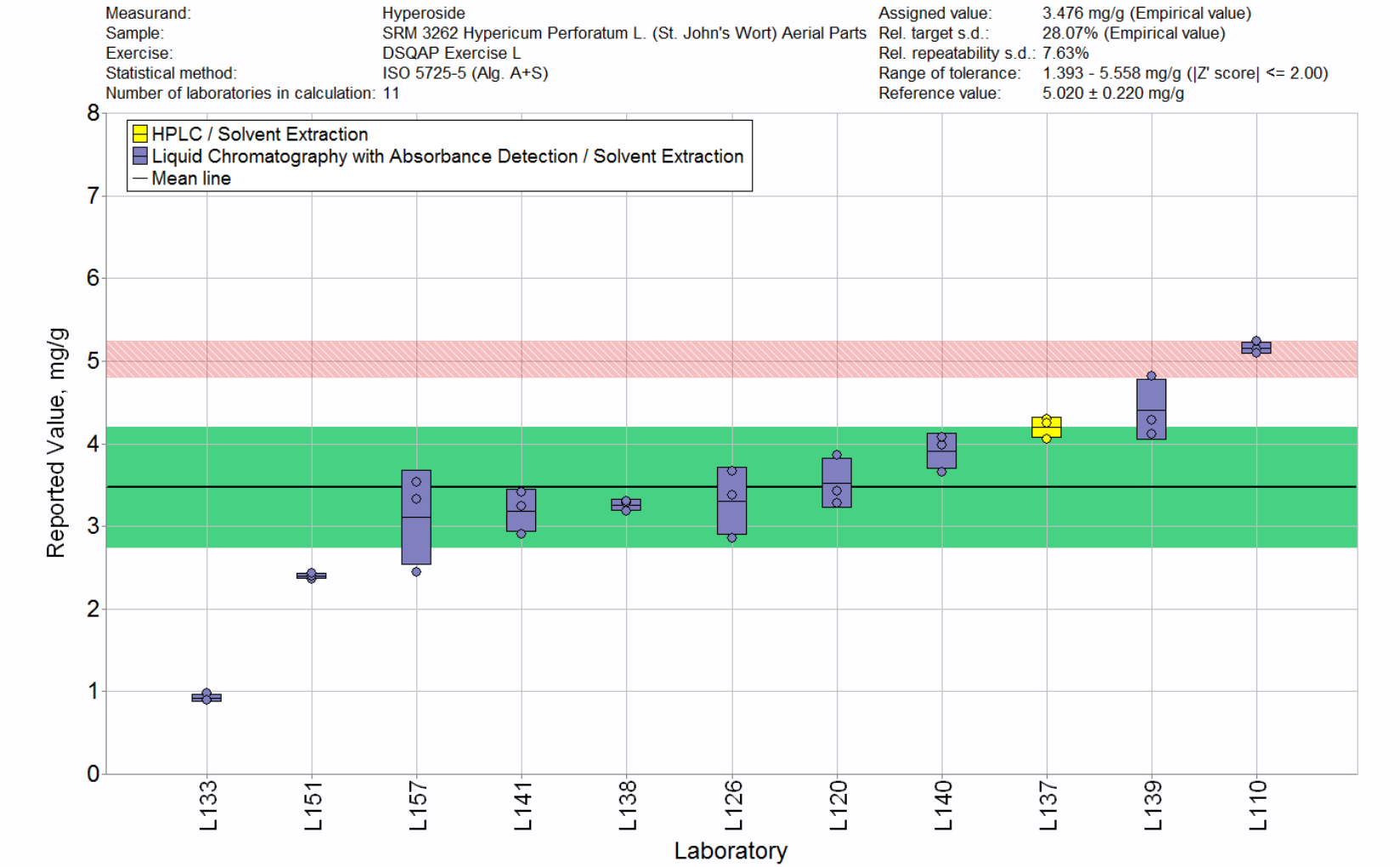

Figure 41. Hyperoside in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

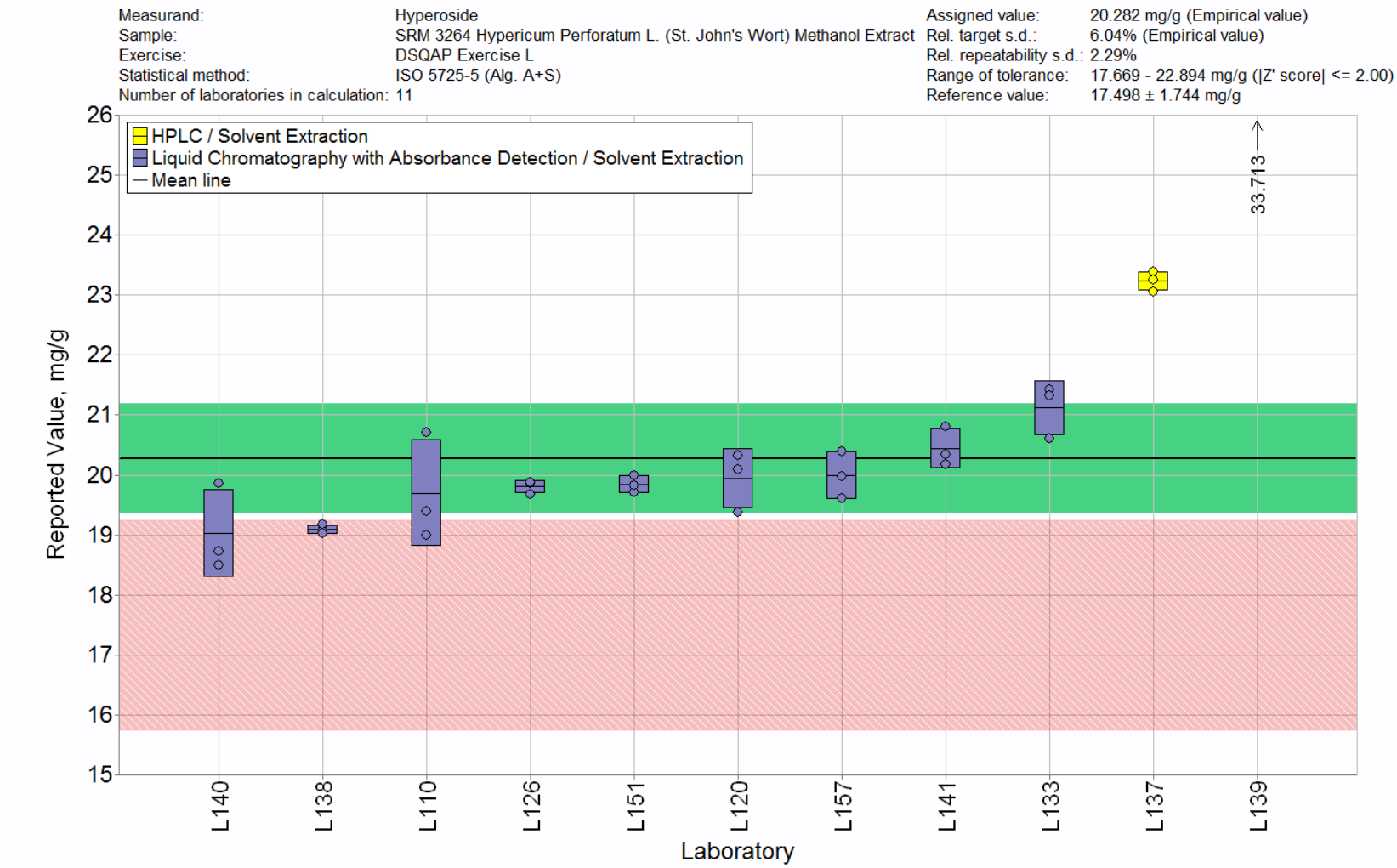

Figure 42. Hyperoside in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

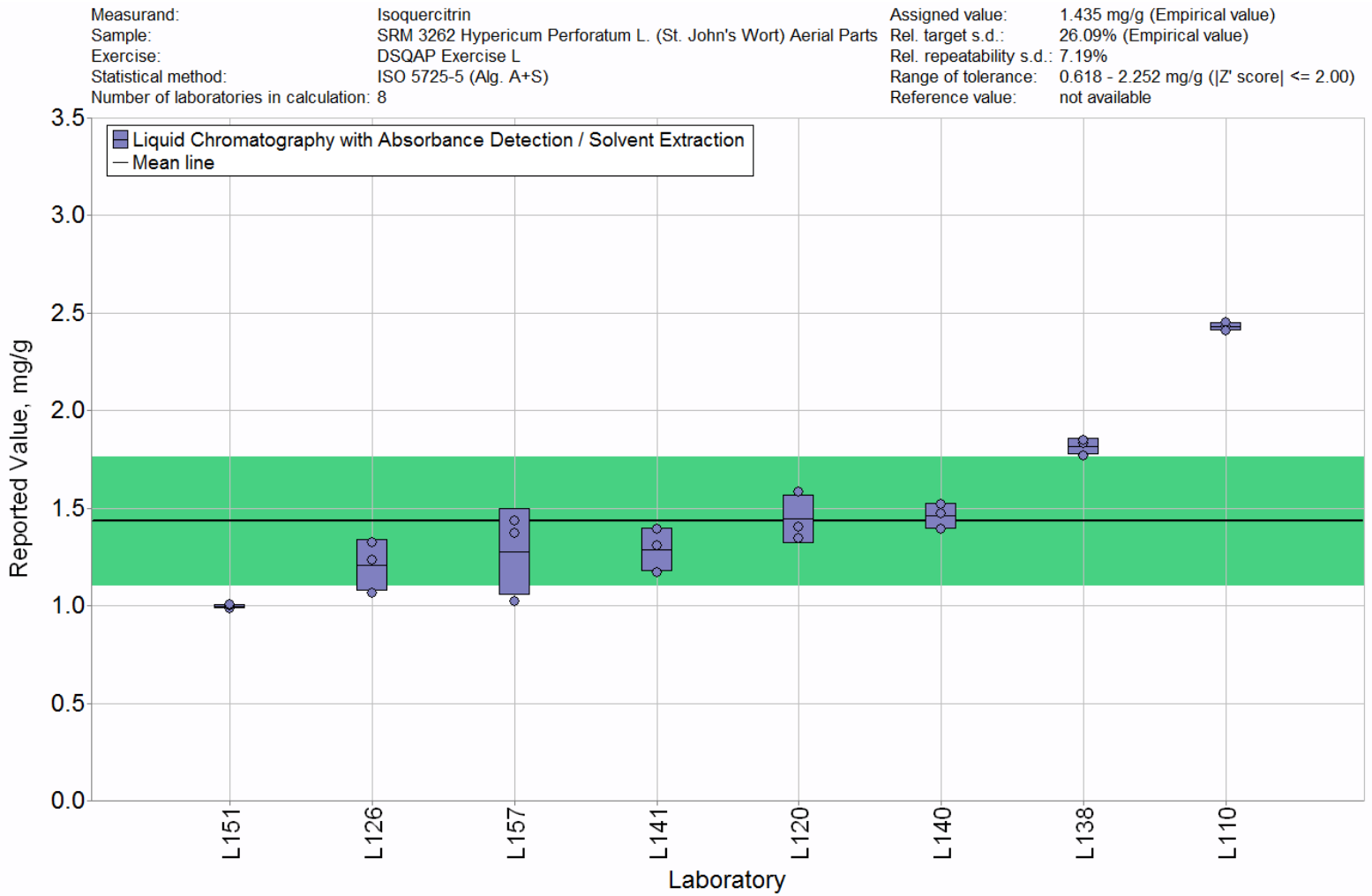

Figure 43. Isoquercitrin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. No NIST-determined value is available for this sample.

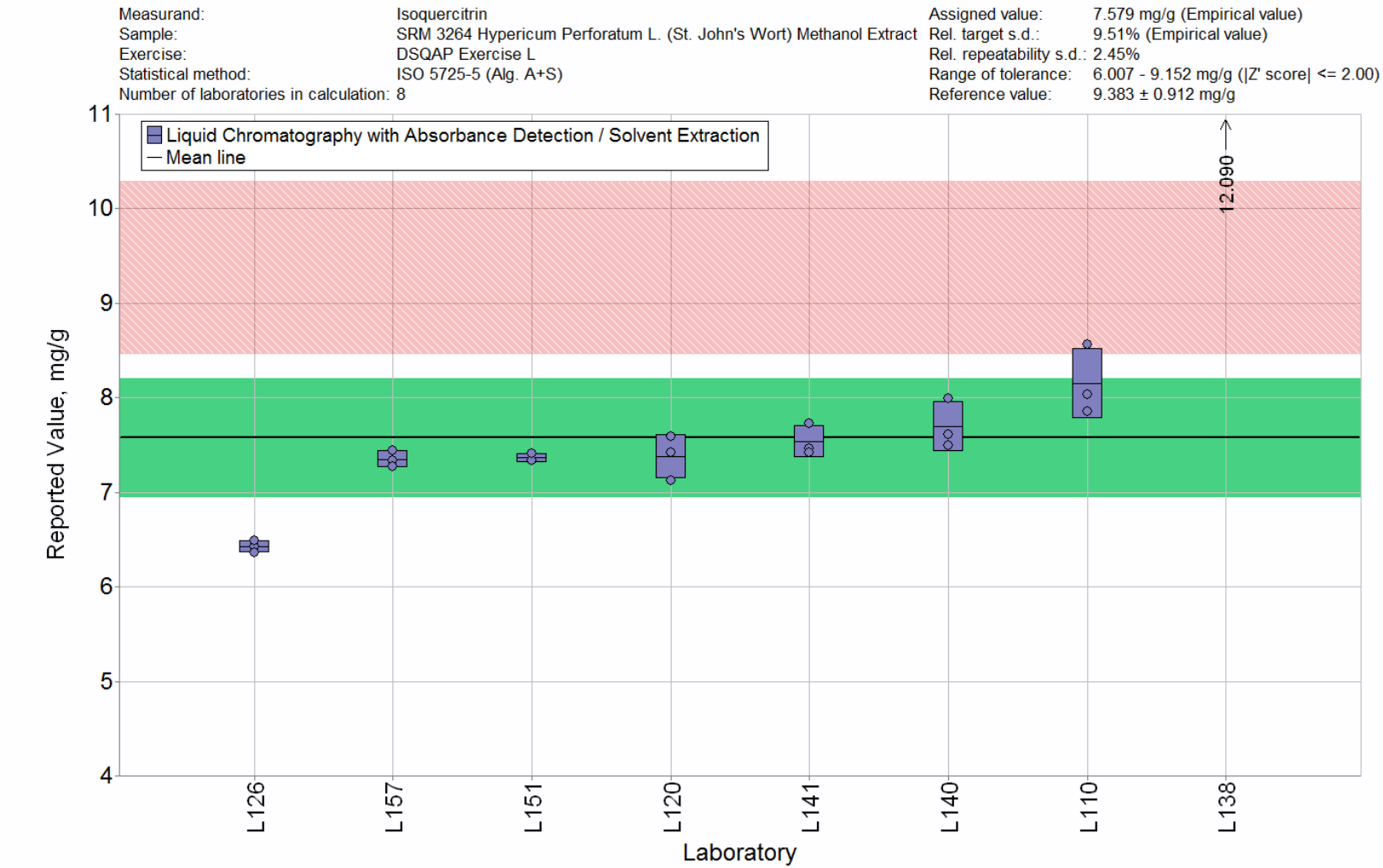

Figure 44. Isoquercitrin in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

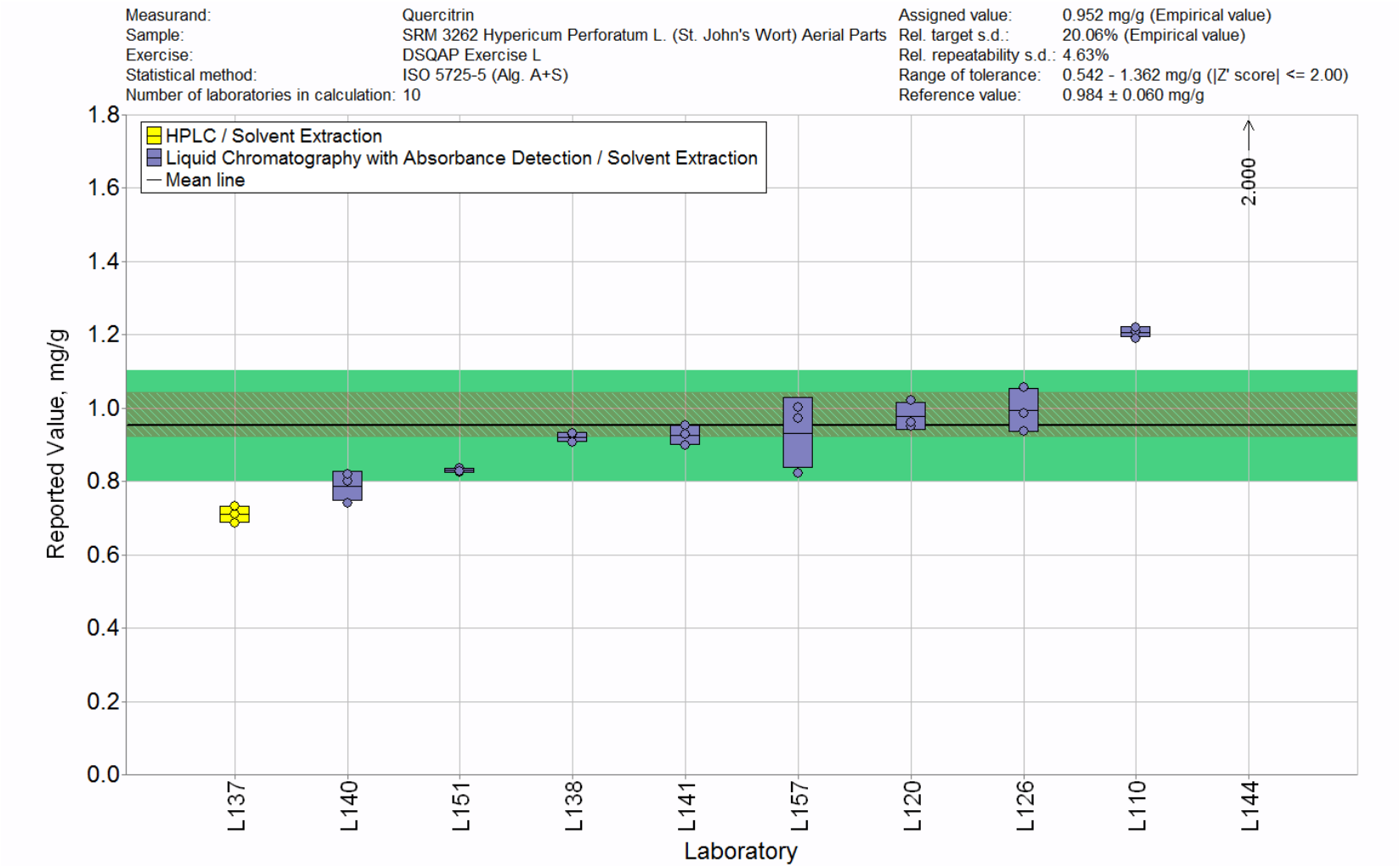

Figure 45. Quercitrin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

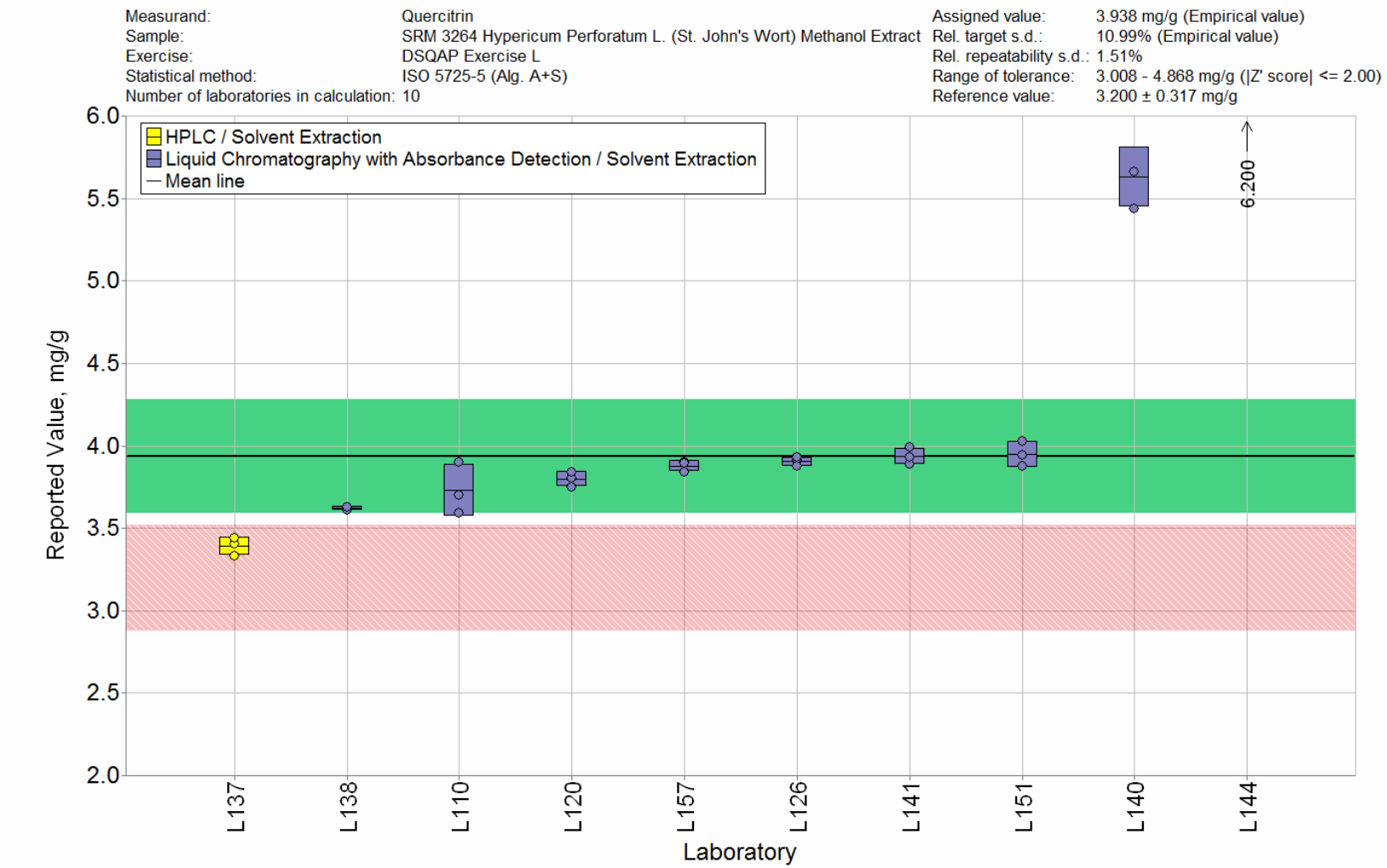

Figure 46. Quercitrin in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

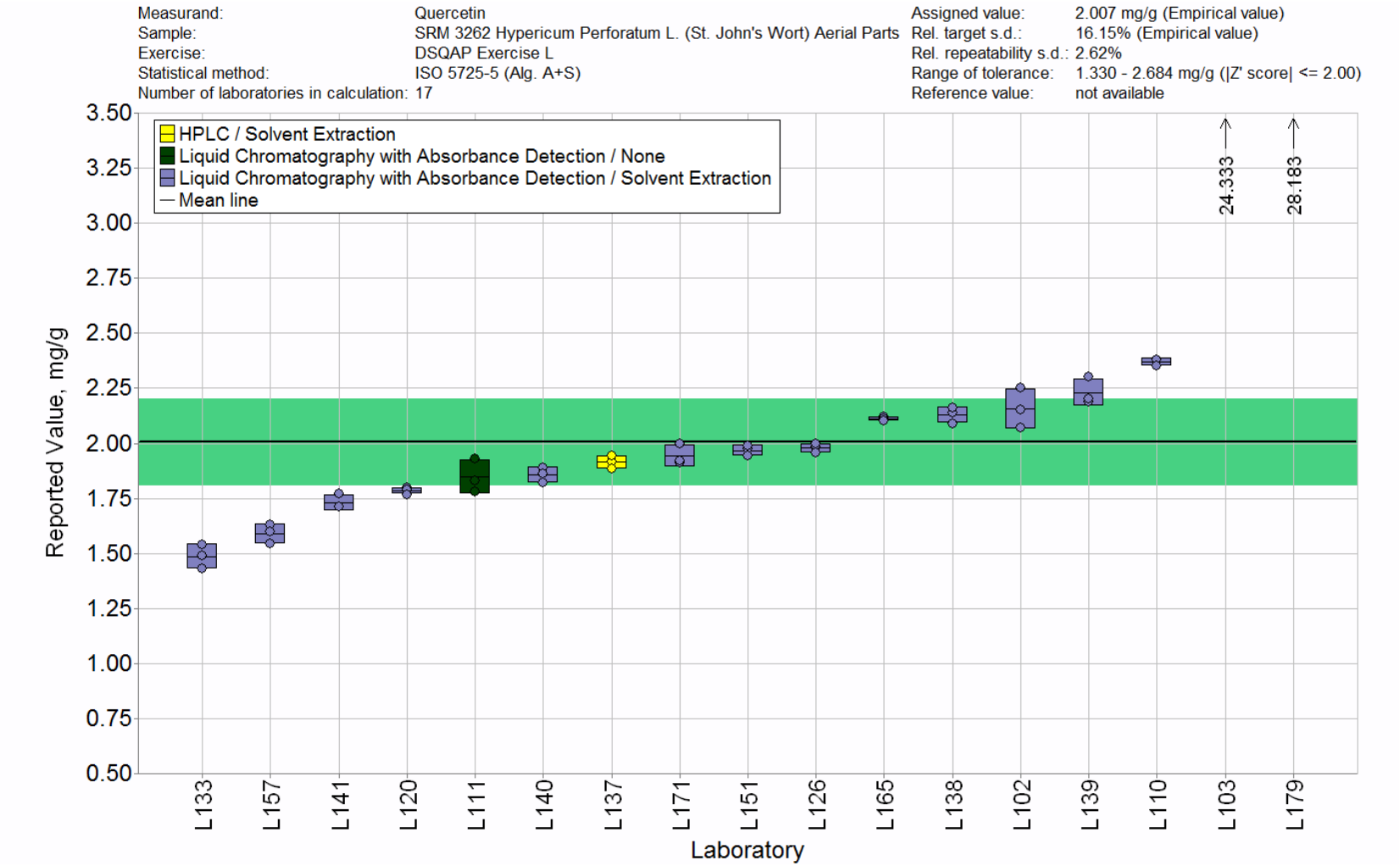

Figure 47. Quercetin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. No NIST-determined value is available for this sample.

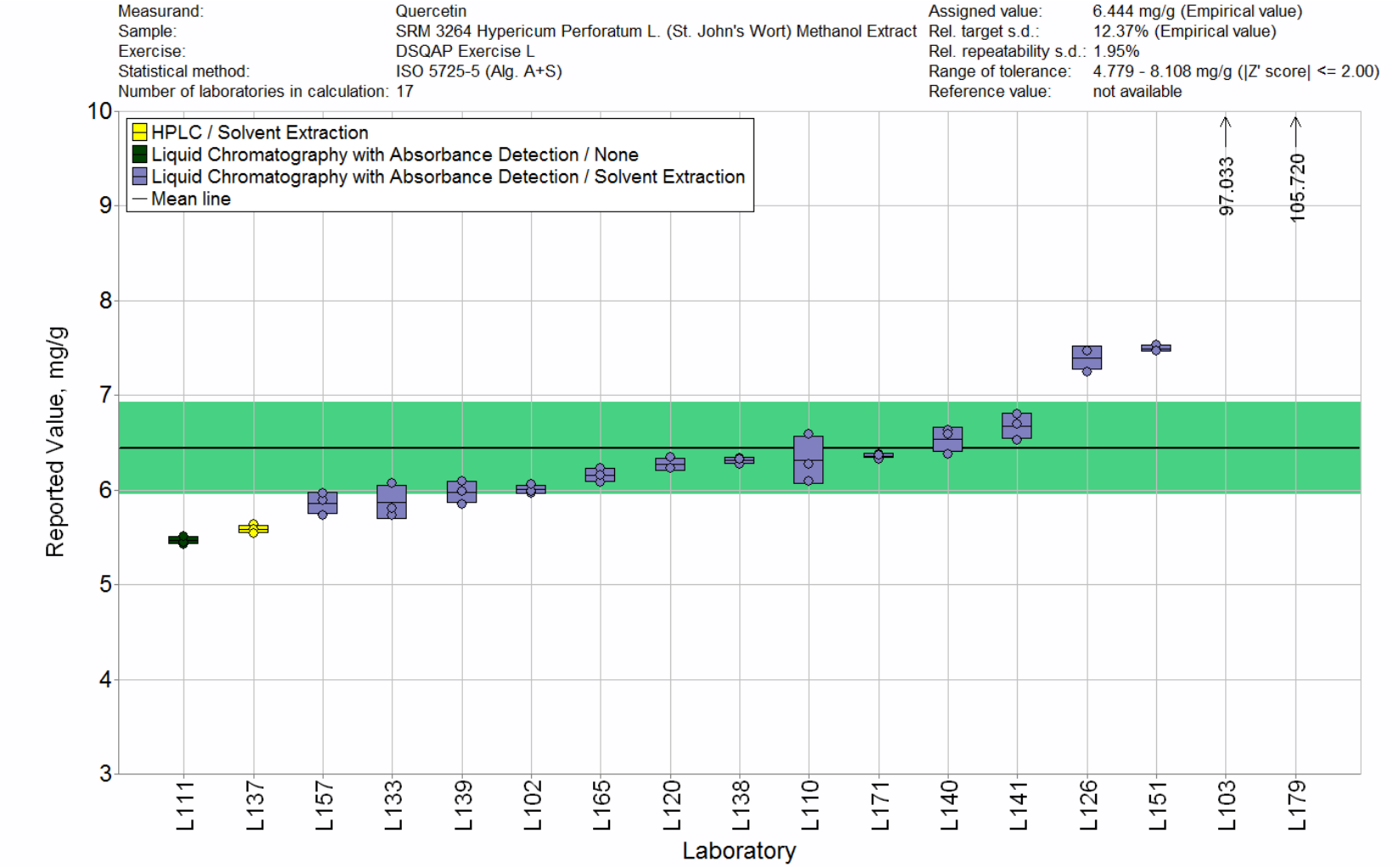

Figure 48. Quercetin in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. No NIST-determined value is available for this sample.

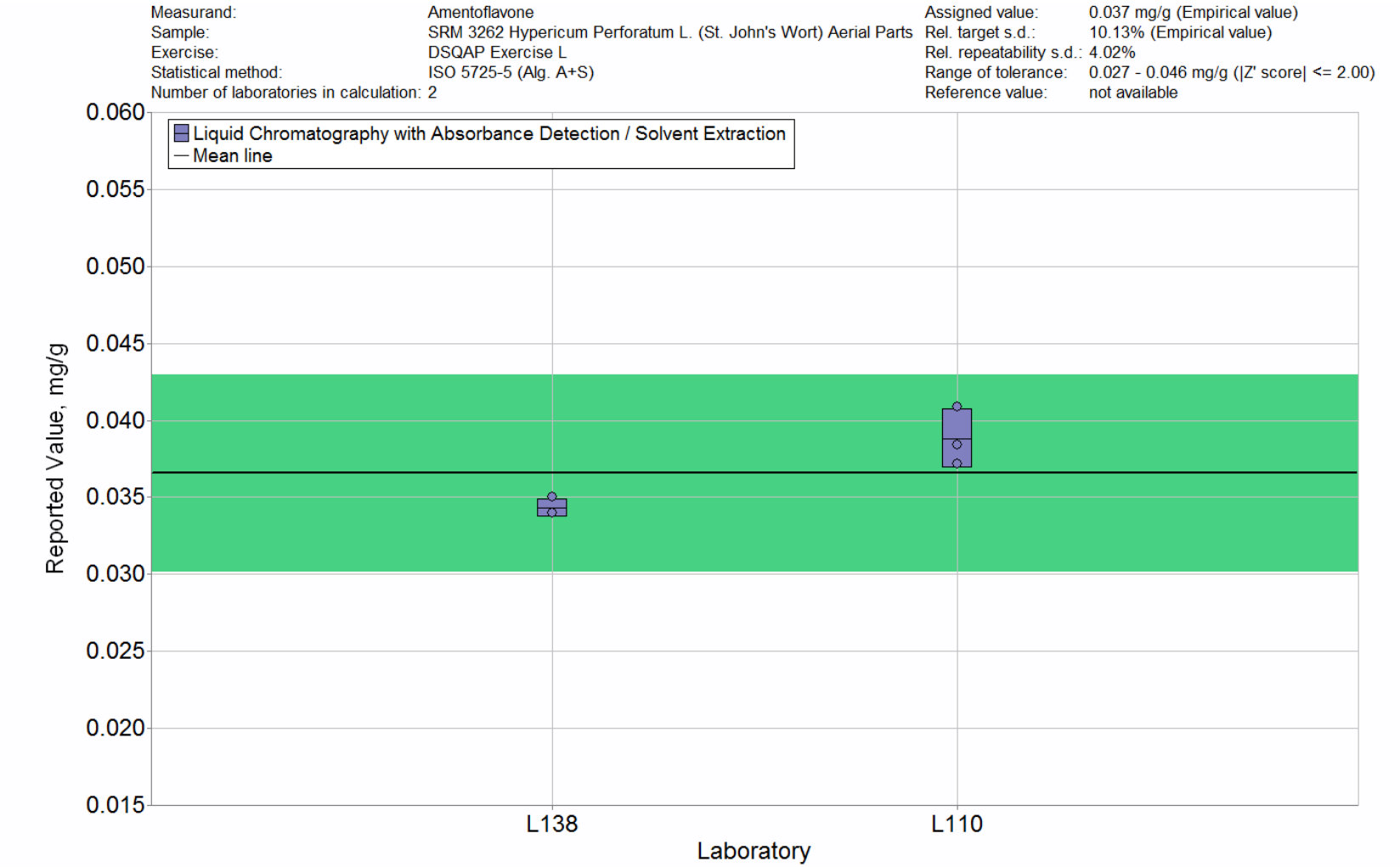

Figure 49. Amentoflavone in St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. No NIST-determined value is available for this sample.

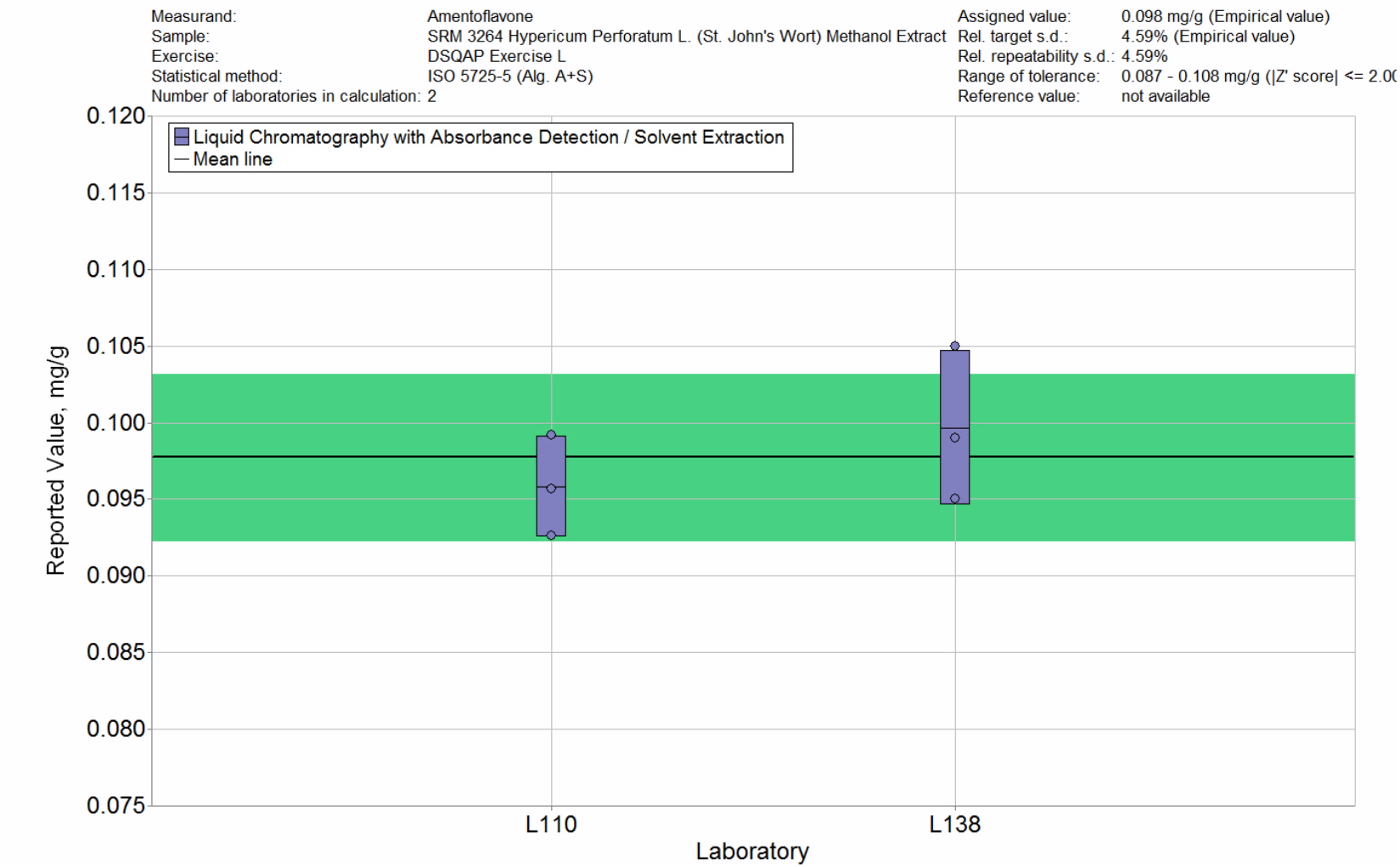

Figure 50. Amentoflavone in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. No NIST-determined value is available for this sample.

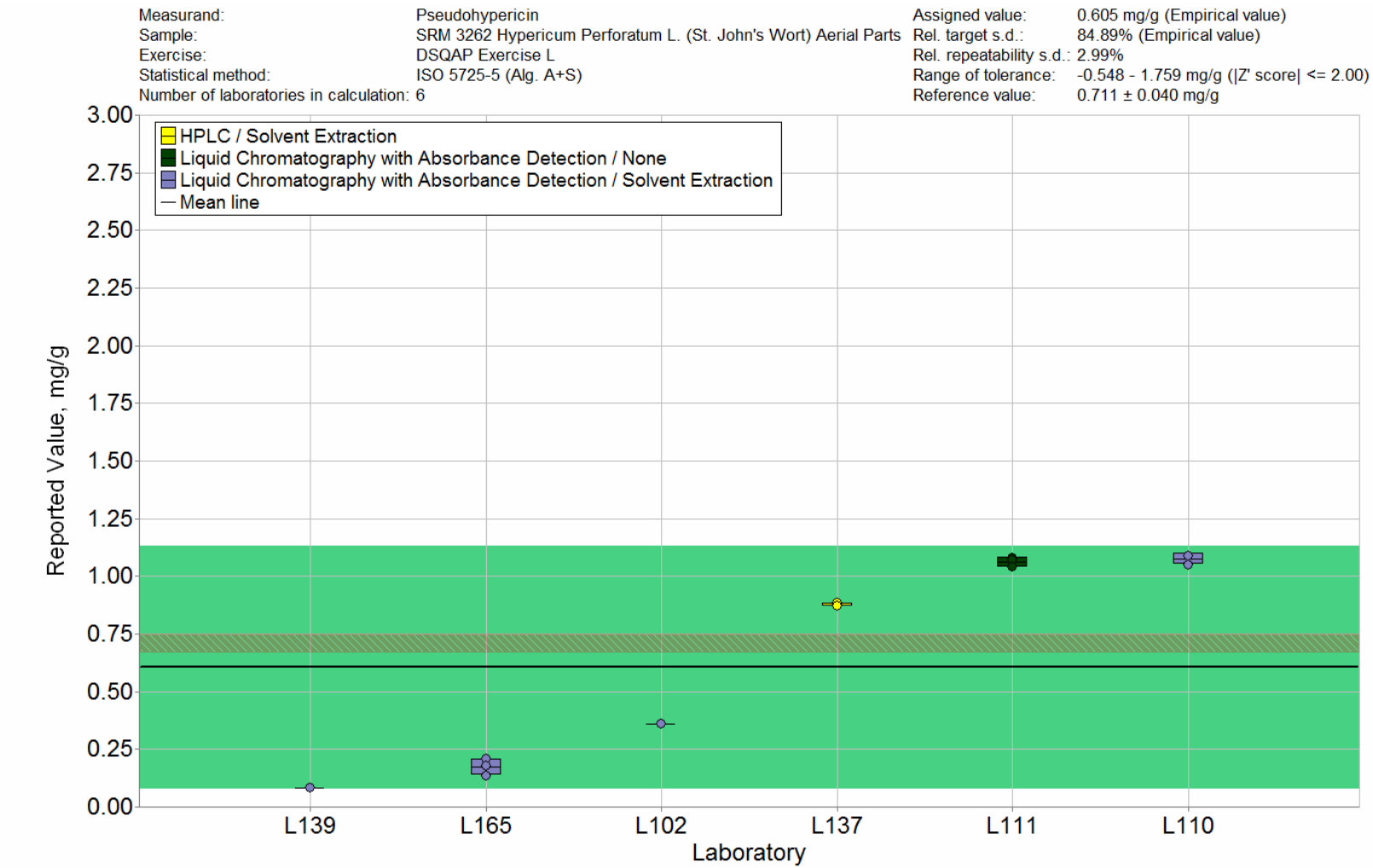

Figure 51. Pseudohypericin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

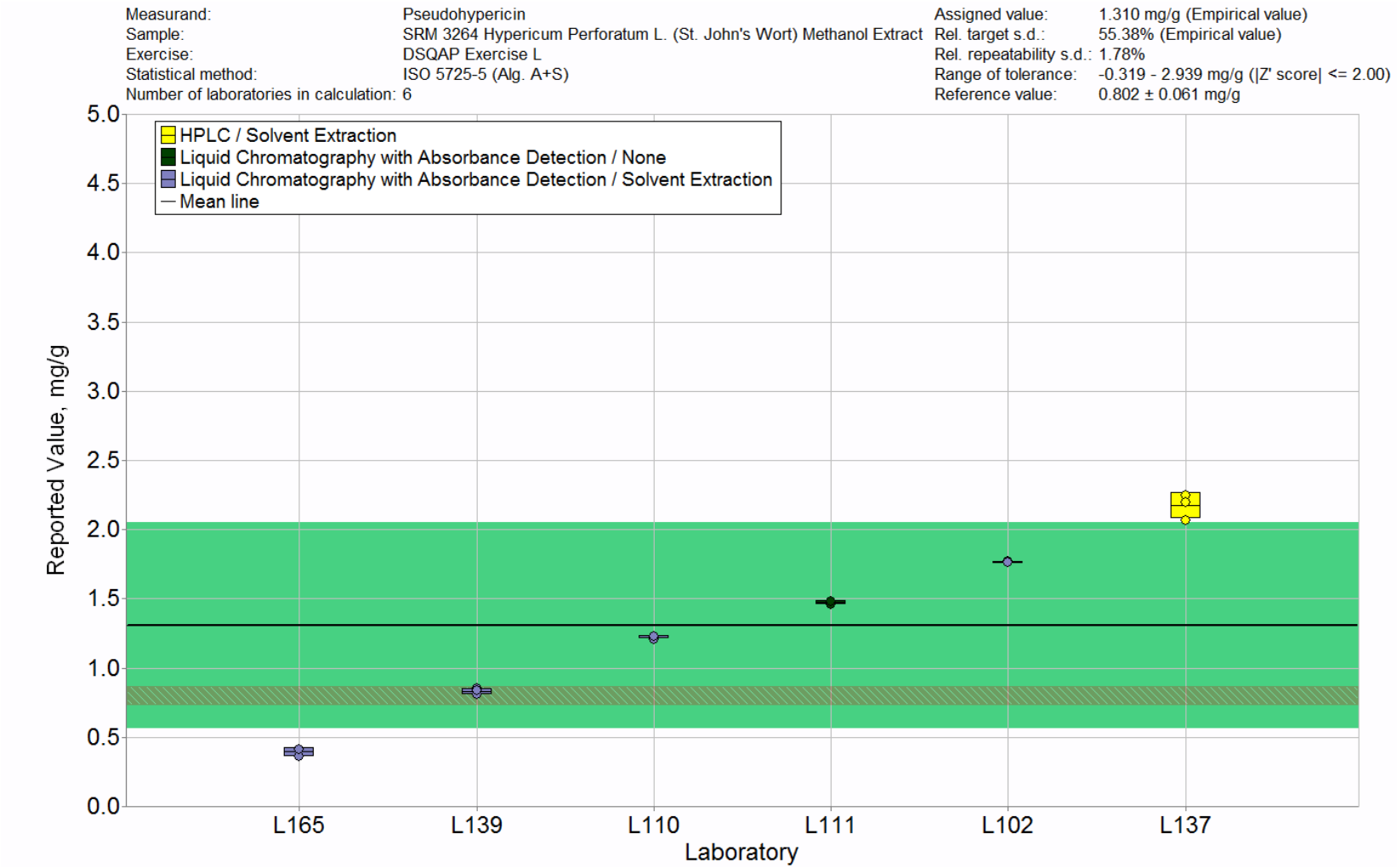

Figure 52. Pseudohypericin in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

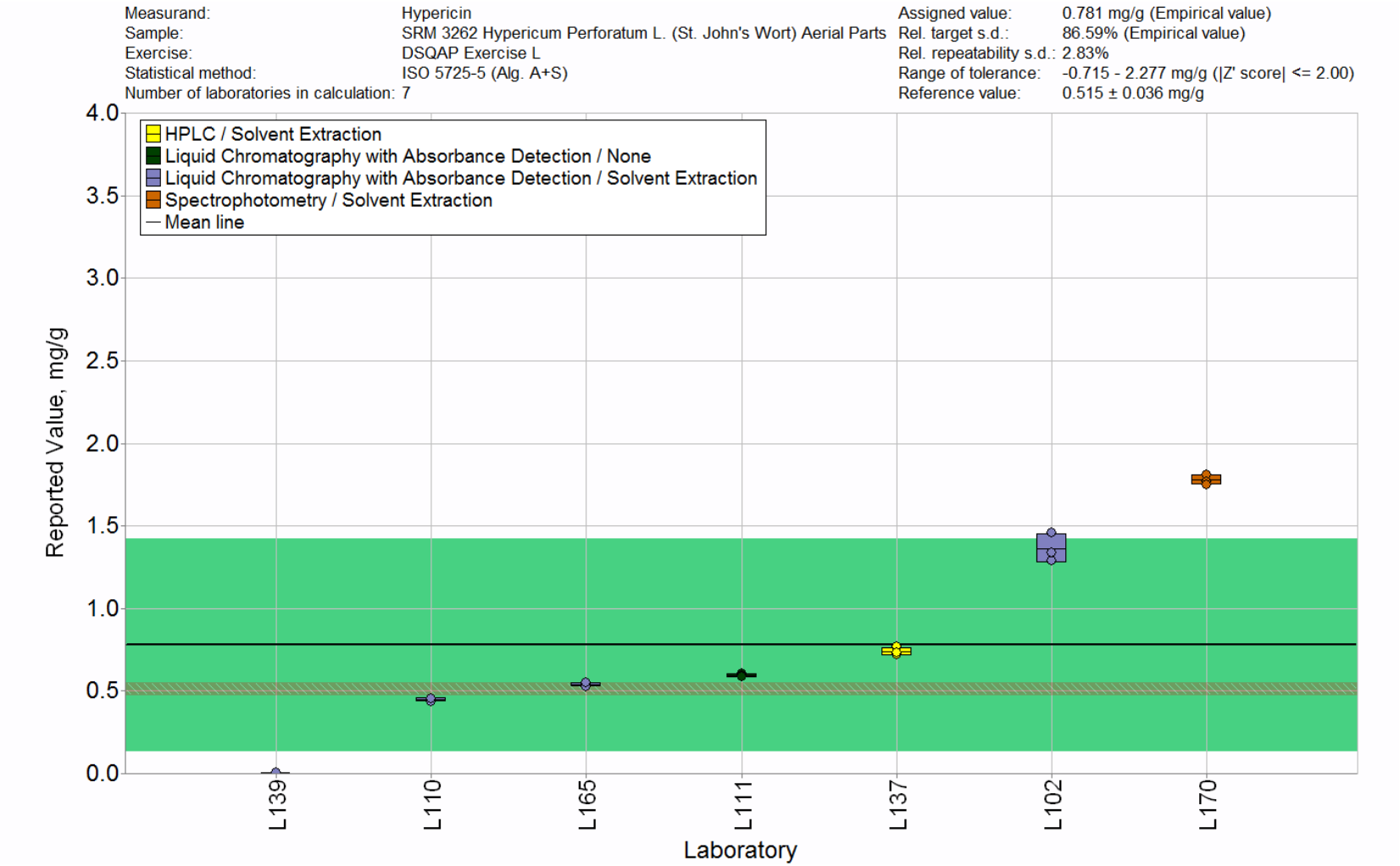

Figure 53. Hypericin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST-determined value bounded by twice its uncertainty (U_{95}).

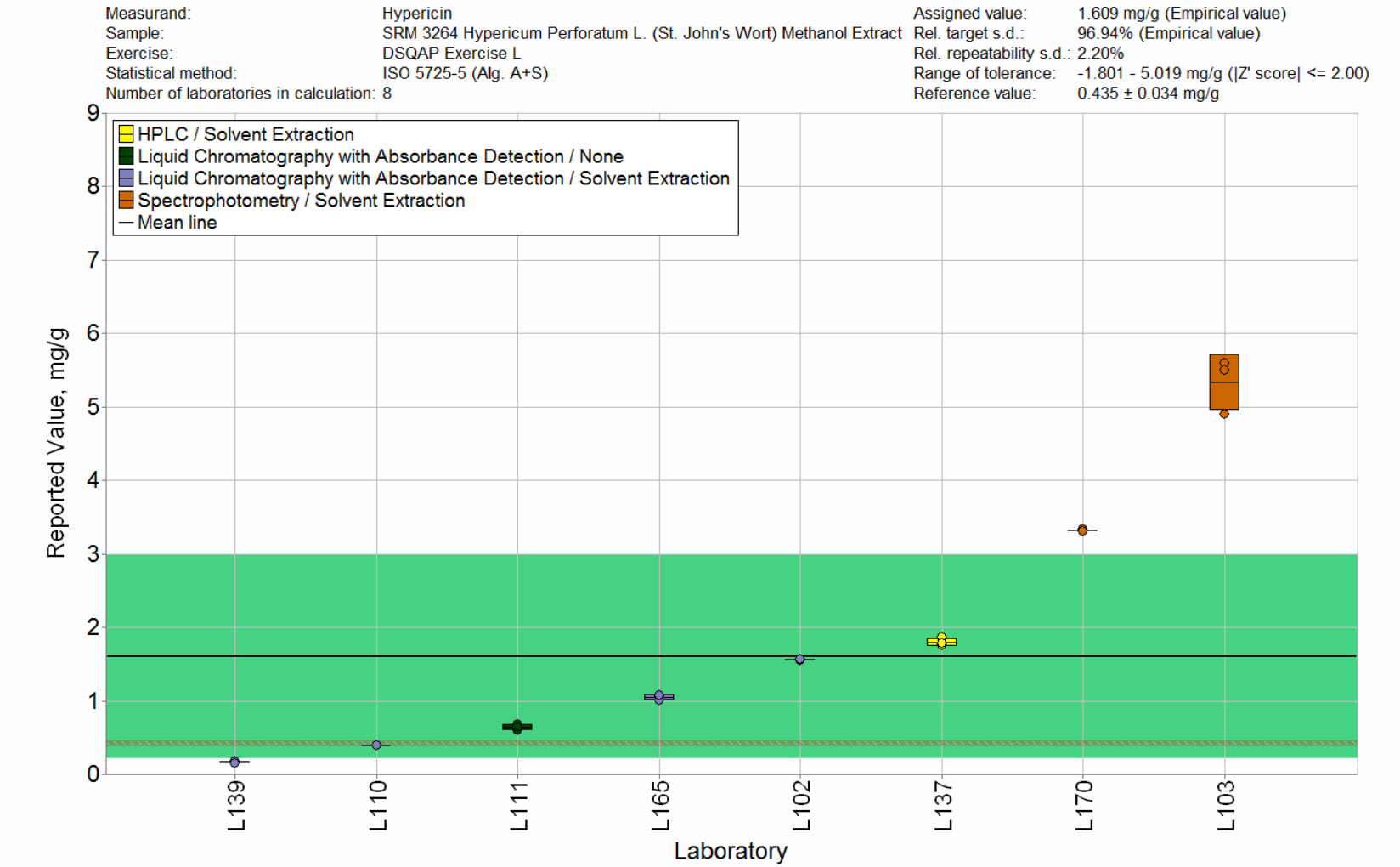

Figure 54. Hypericin in SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (data summary view – sample preparation and analytical method). In this view, individual laboratory data are plotted (circles) with the individual laboratory standard deviation (rectangle). The color of the data point represents the sample preparation procedure and analytical method employed. The black solid line represents the consensus mean, and the green shaded region represents the range of tolerance, calculated as the values above and below the consensus mean that result in an acceptable Z' score, $|Z'| \leq 2$. The red shaded region represents the target zone for "acceptable" performance, which encompasses the NIST reference value bounded by twice its uncertainty (U_{95}).

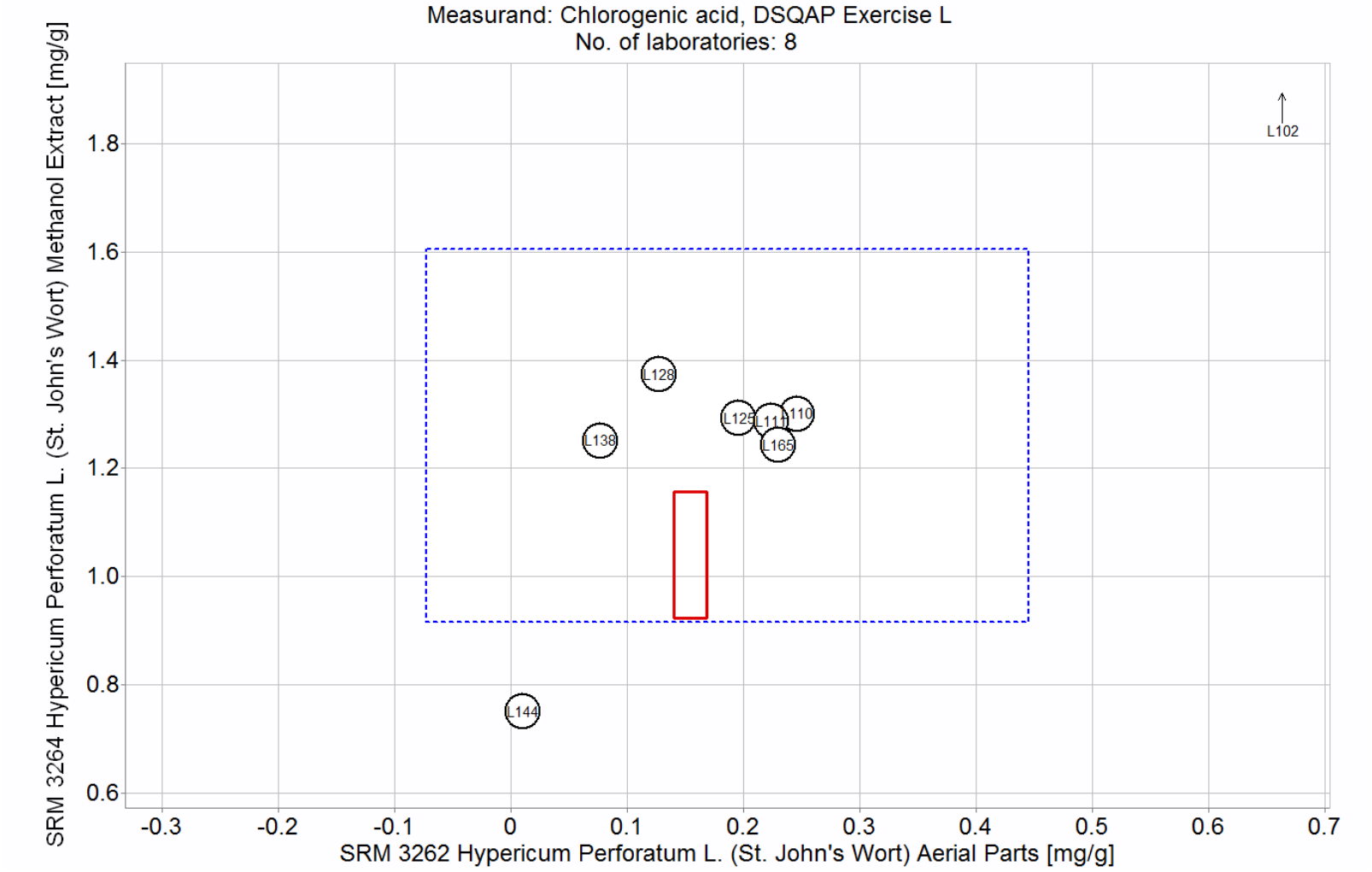

Figure 55. Chlorogenic acid in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The solid red box represents the target zone for the two samples, St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

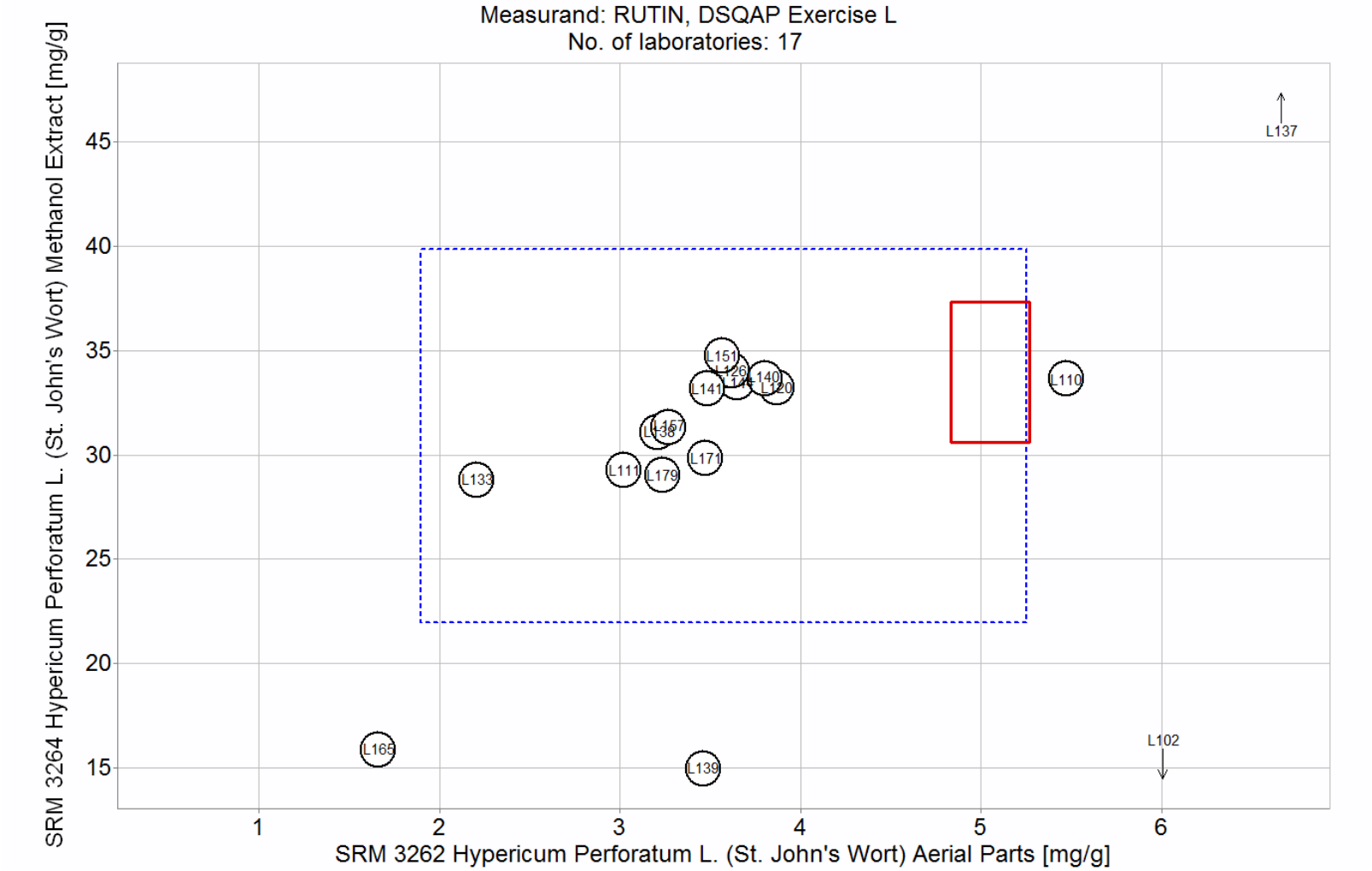

Figure 56. Rutin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The solid red box represents the target zone for the two samples, St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

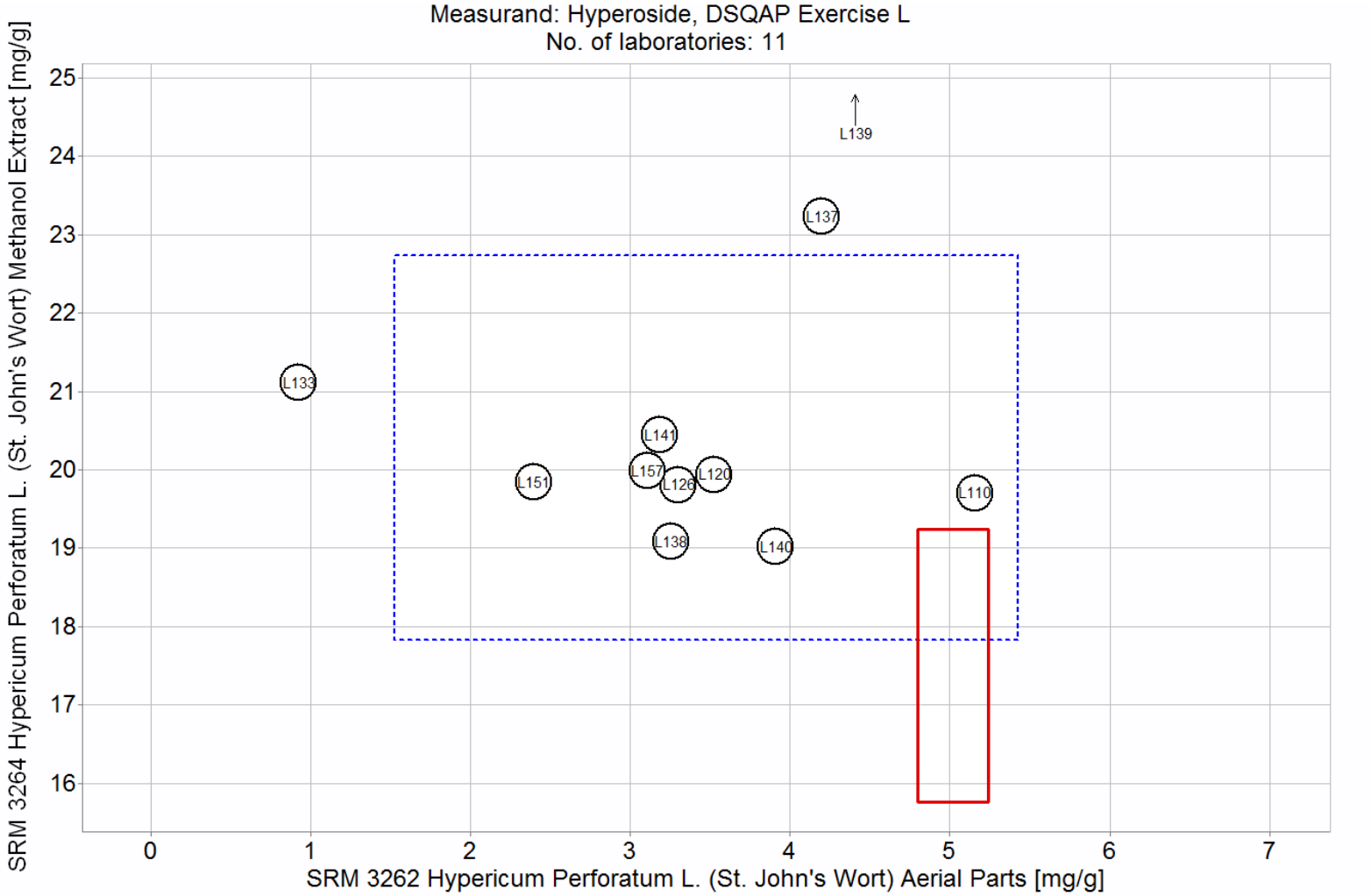

Figure 57. Hyperoside in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The solid red box represents the target zone for the two samples, St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

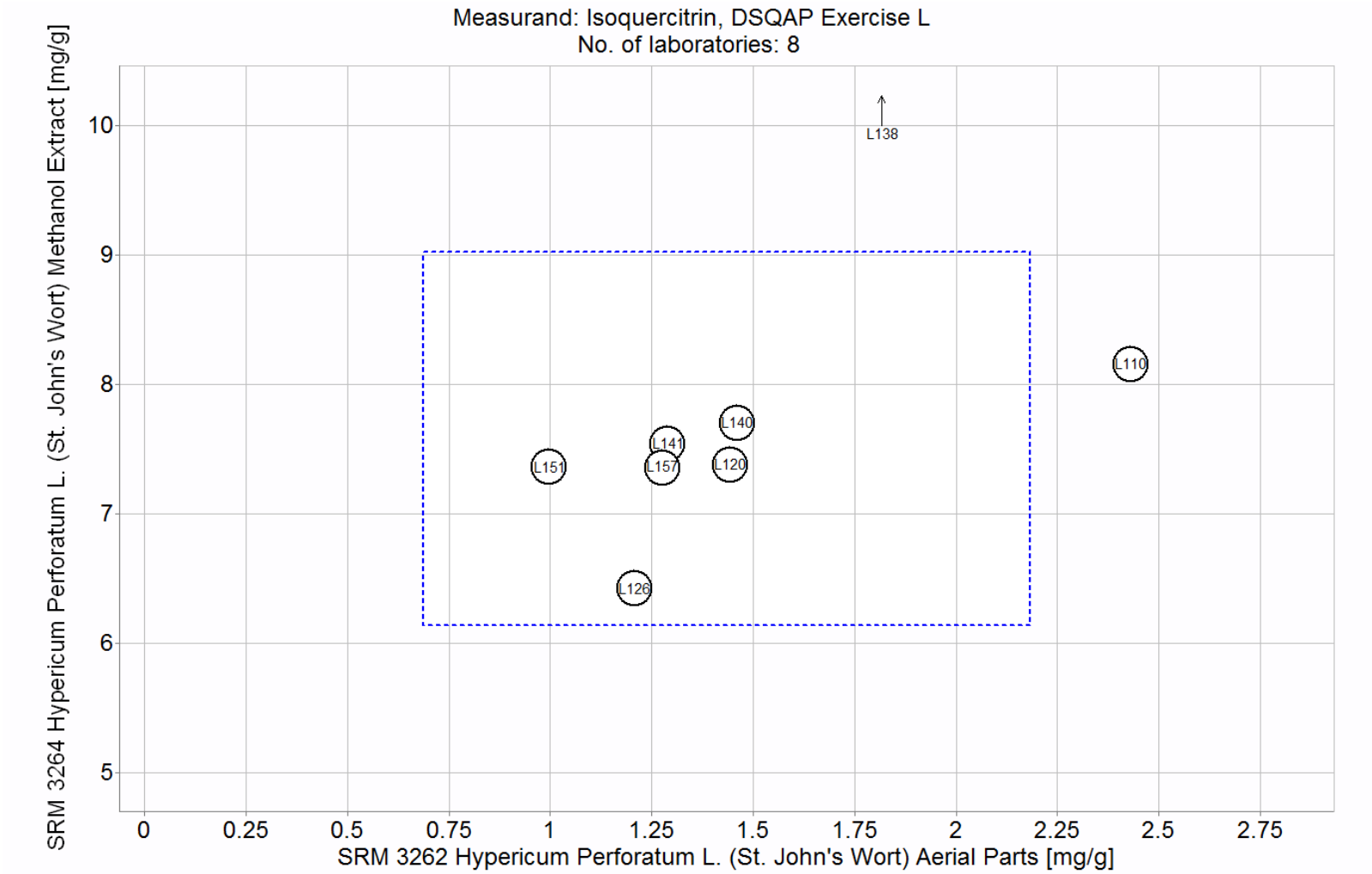

Figure 58. Isoquercitrin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

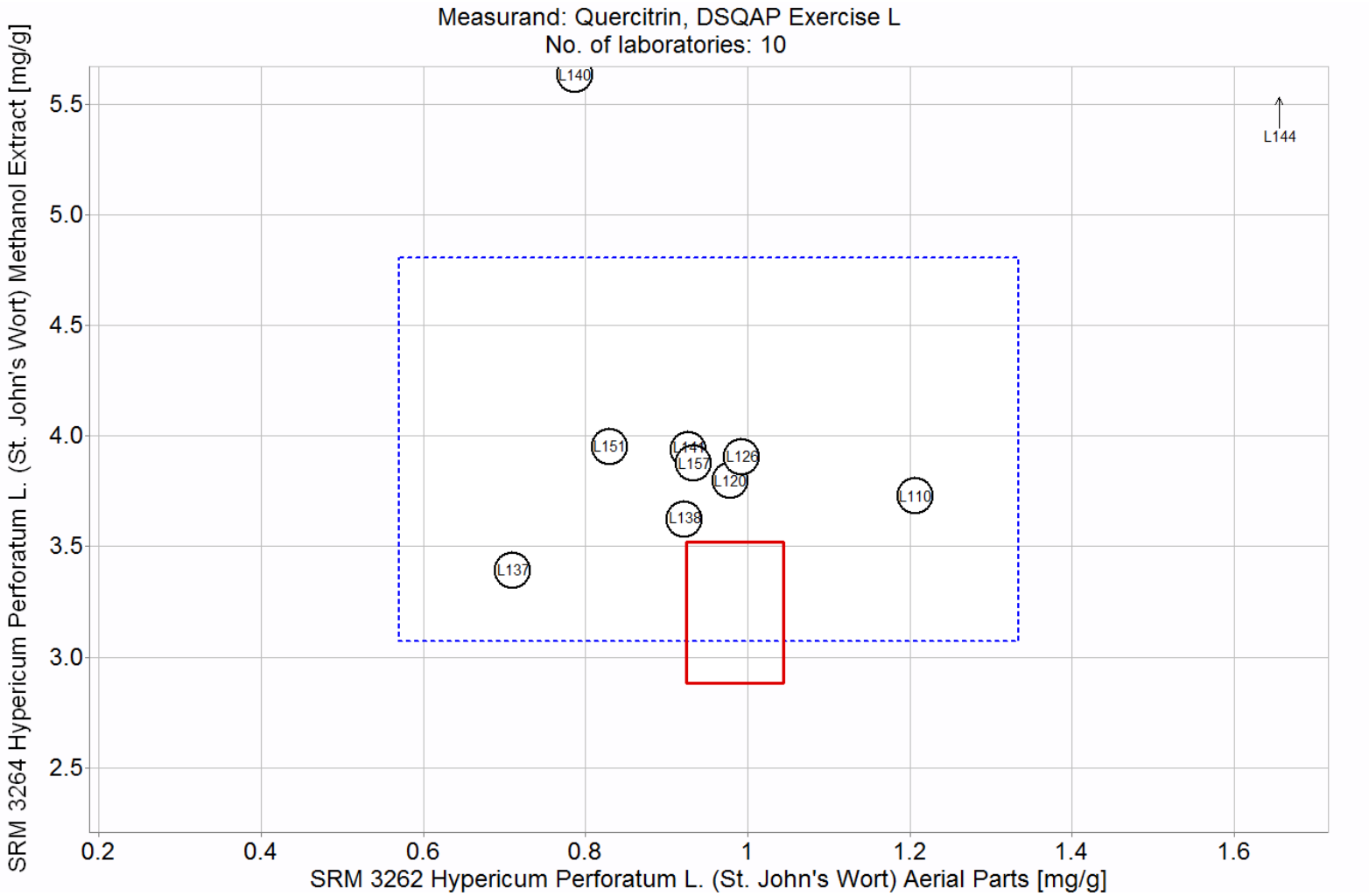

Figure 59. Quercitrin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The solid red box represents the target zone for the two samples, St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

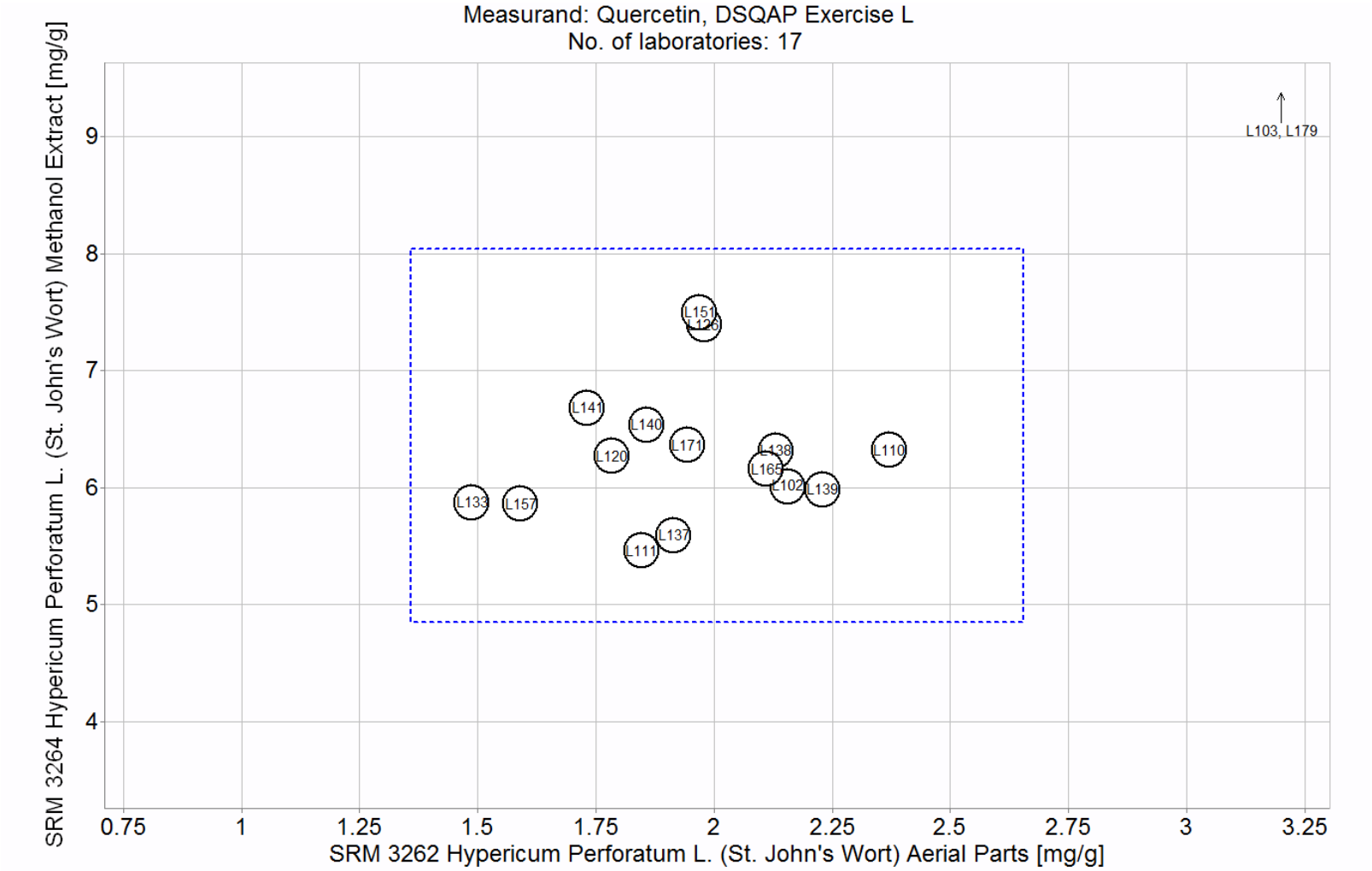

Figure 60. Quercetin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

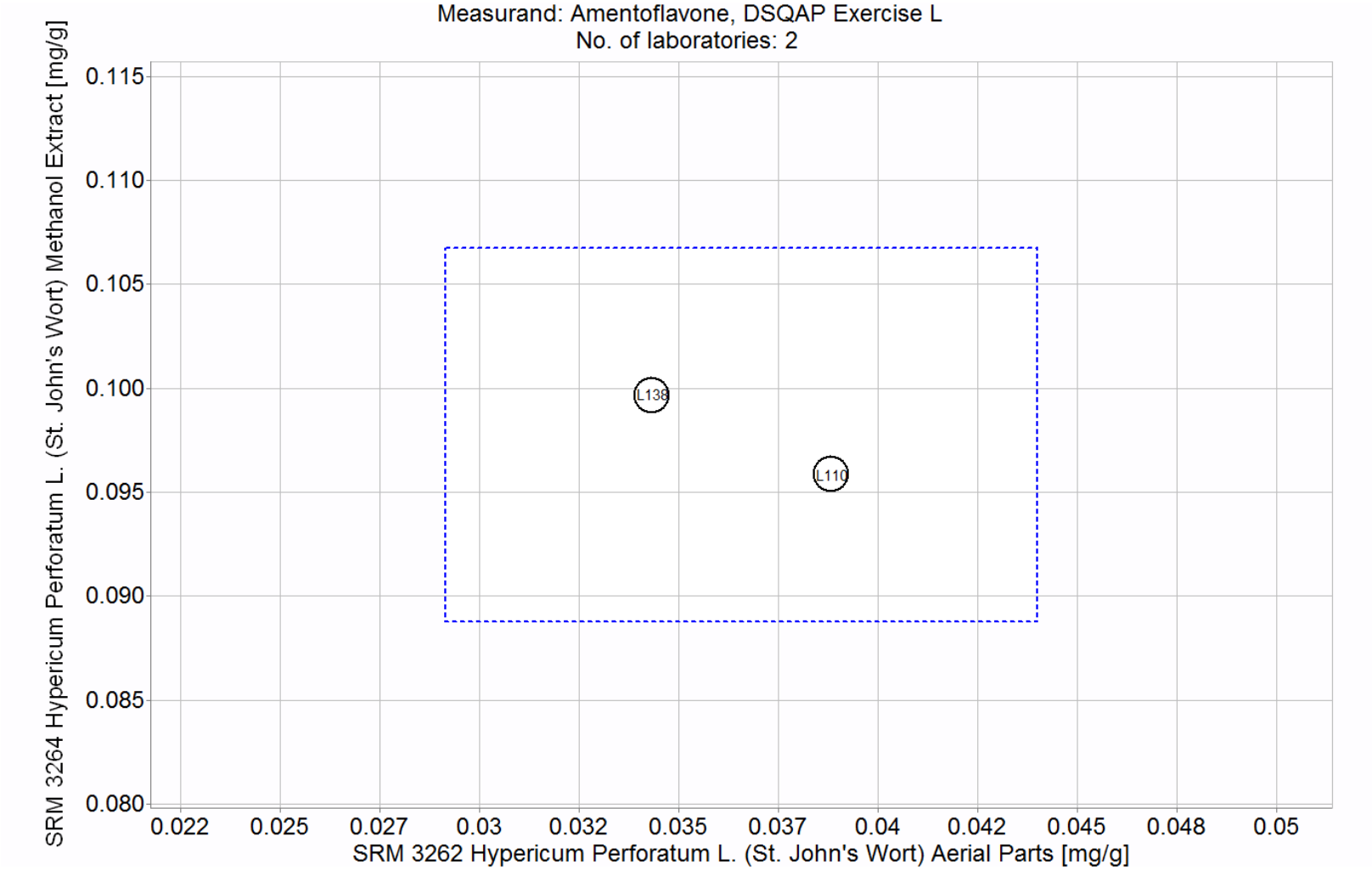

Figure 61. Amentoflavone in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

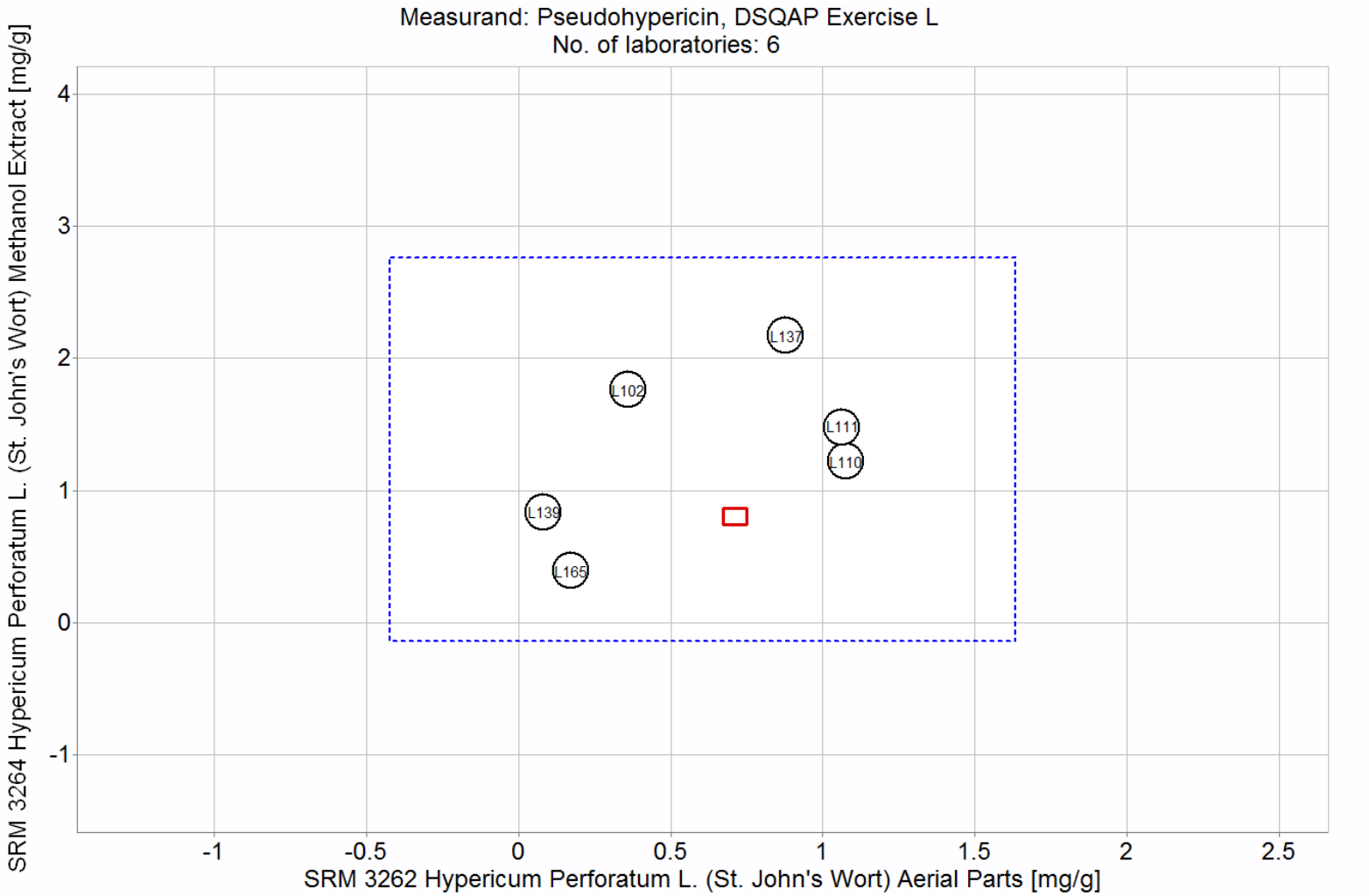

Figure 62. Pseudohypericin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The solid red box represents the target zone for the two samples, St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).

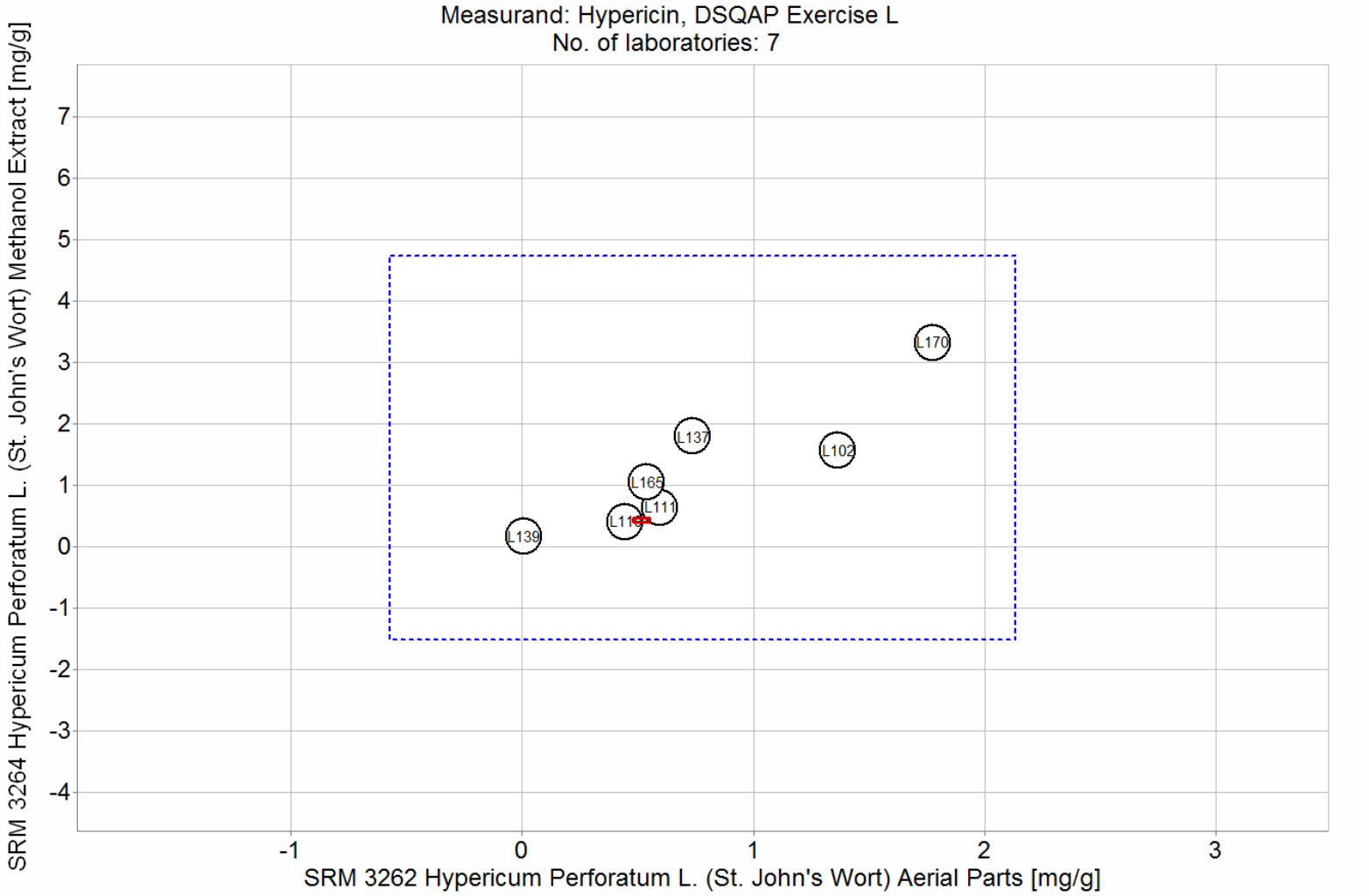

Figure 63. Hypericin in SRM 3262 St. John's Wort (*Hypericum perforatum L.*) Aerial Parts and SRM 3264 St. John's Wort (*Hypericum perforatum L.*) Methanol Extract (sample/sample comparison view). In this view, the individual laboratory results for one sample (St. John's wort aerial parts) are compared to the results for a second sample (St. John's wort methanol extract). The solid red box represents the target zone for the two samples, St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis). The dotted blue box represents the consensus zone for St. John's wort aerial parts (x-axis) and St. John's wort methanol extract (y-axis).