Dear DSQAP participants,

Thank you for your participation in Exercise D of the Dietary Supplement Laboratory Quality Assurance Program. The attached report contains four sections: an overview of the statistical analysis; your individual data table; graphs displaying the community performance; and general recommendations from the exercise. A participation certificate will be sent in a separate e-mail message.

Please check your laboratory's data in the table and make sure you agree with it. If you do not, please let us know so that we can correct it.

We are planning to hold an analyst workshop at NIST in September 2010. Participants in exercises C, D, and E will receive further information about the workshop in the early summer.

If you have any questions or suggestions, please let us know.

Best regards,
DSQAP Team

Dietary Supplement Quality Assurance Program
Exercise D
Final Data Report

Your laboratory code for this exercise: NIST

This report consists of several parts:

- **Overview**: a general description of the statistical treatment of the data and how to read the plots.

- **Data Table**: a table with your laboratory's individual results, the community results, and the NIST results.

- **Graphs**: a section that includes graphical representation of the data for the analytes tested in this exercise, points to consider when examining the data, and when appropriate, recommendations from Exercise D.
  - Lead
  - Niacin
  - β-carotene
  - Organic acids

- **Recommendations**

As always, if you have any questions, please contact us.

OVERVIEW

STATISTICS

Your individual data table and graphs contain information about your performance relative to that of the other participants in this exercise and relative to a target around the expected result.

INDIVIDUAL DATA TABLE

Section 1 of the data table contains your results, including your mean, standard deviation, and Z-score. Please check these and make sure that you agree with the mean and standard deviation reported in the table. The significance of the Z-score is as follows:

- $|Z| < 2$ indicates that your result is considered to be within the community consensus value
- $2 < |Z| < 3$ indicates that your result is considered to be marginally different from the community consensus value
- $|Z| > 3$ indicates that your result is considered to be significantly different from the community consensus value

Section 2 of the data table contains the community results, including the median value for each analyte, the MADe (a robust estimate of the standard deviation), and the minimum/maximum values reported for the analyte.

Section 3 of the data table contains the NIST results. In most cases, the assigned value and the U_{95} confidence interval have been determined with two independent analytical methods. At least six samples have been tested with each of the methods and duplicate sample preparations from the sample package have been included, allowing the U_{95} to encompass homogeneity within and between packages.

GRAPHS

Two graphs are provided for each analyte in each sample, one which plots the results for the sample only (View 1) and one which plots the lab results for the sample vs. the lab results for the control (View 2). Both views include the consensus values and the target values.

View 1

In this view, individual laboratory data are plotted with the individual laboratory standard deviation. The black solid line represents the consensus median and the black dotted lines represent the consensus variability calculated as 2*MADe. The center of the region between the red lines represents the NIST view of the "correct answer;" it is bounded by two times the pooled standard deviation ($2*S_{total, pooled}$) of the participants or the NIST uncertainty (U_{95}) (whichever is larger), thus creating a target zone for "acceptable" performance. With this view, it is relatively easy to determine if a laboratory falls within

the target zone and to compare where the target zone lies relative to the consensus values. In most cases, the target zone falls within the consensus values, which is the expected result. One program goal is to bring the consensus values closer together and closer to the target value.

View 2

In this view, the results reported for the sample are plotted vs. the results for the control. The red box represents the target values for the control (x-axis) and the sample (y-axis) and the blue dotted lines represent the analogous information for the consensus values.

This view provides additional information to complement view 1. For example, if your values are low (or high) for both the control and sample, you may have calibration issues. If your laboratory falls into this category, you may want to investigate how your calibrants are prepared as well as the purity of your calibrant material.

Return to Introduction

National Institute of Standards and Technology

Lab Code:	NIST	Your Results			Community Results					NIST Results	
Analyte	Units	Mean	s_{total}	Z	N	Median	MADe	Min	Max	Value	U_{95}
Niacin	µg/g	106.7	3.4	-0.4	48	107.67	2.295	93.13	149.33	106.7	3.4
Total b-carotene	µg/g	35.5	8.3	5.9	32	22.78	2.145	10.97	54.40	35.5	8.3
9-cis-β-carotene	µg/g	13.9	4.4	5.6	14	8.63	0.947	3.34	15.75	13.9	4.4
all-trans-β-carotene	µg/g	21.4	5.0	8.6	17	11.78	1.115	7.63	21.20	21.4	5.0
Quinic Acid	mg/g	12.2	1.5	-1.4	27	12.52	0.227	5.37	20.90	12.2	1.5
Malic Acid	mg/g	5.90	1.20	4.0	27	5.22	0.169	3.73	24.13	5.90	1.20
Citric Acid	mg/g	22.9	1.8	2.9	27	21.27	0.562	14.63	38.13	22.9	1.8
Lead	µg/g	0.7753	0.0089	3.0	51	0.72	0.017	0.44	1.01	0.7753	0.0089

Mean Average of reported values

s_{total} Standard deviation of reported values

Z Z-score: (Mean - Median)/MADe

N Number of quantitative values reported

Median Median of the reported values

MADe robust estimate of the standard deviation derived from the median absolute deviation (MAD)

Min Minimum reported value

Max Maximum reported value

Value NIST-assessed value

U_{95} ±95% confidence interval about the assessed value

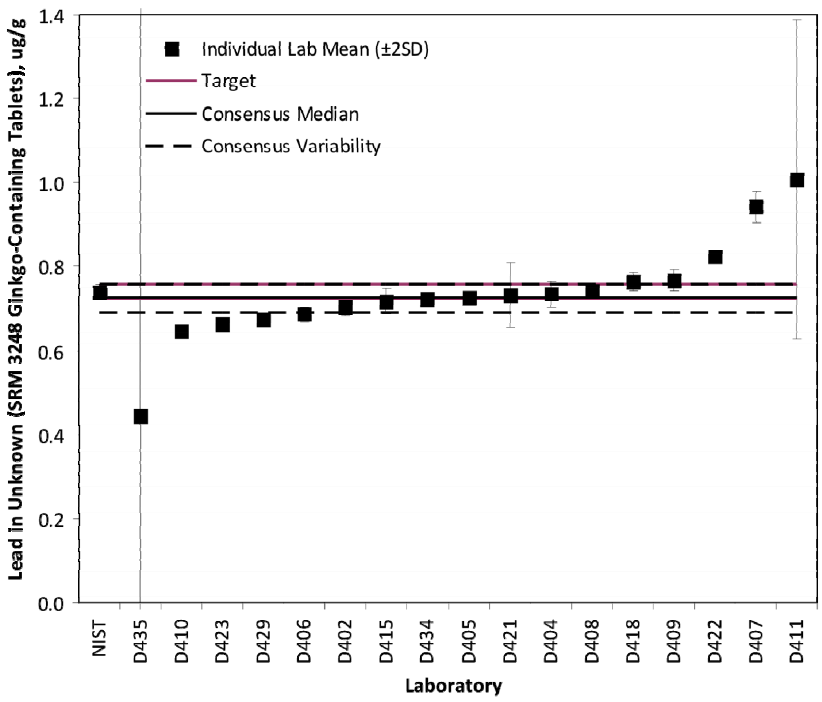
Return to Introduction

LEAD IN GINKGO-CONTAINING PRODUCTS

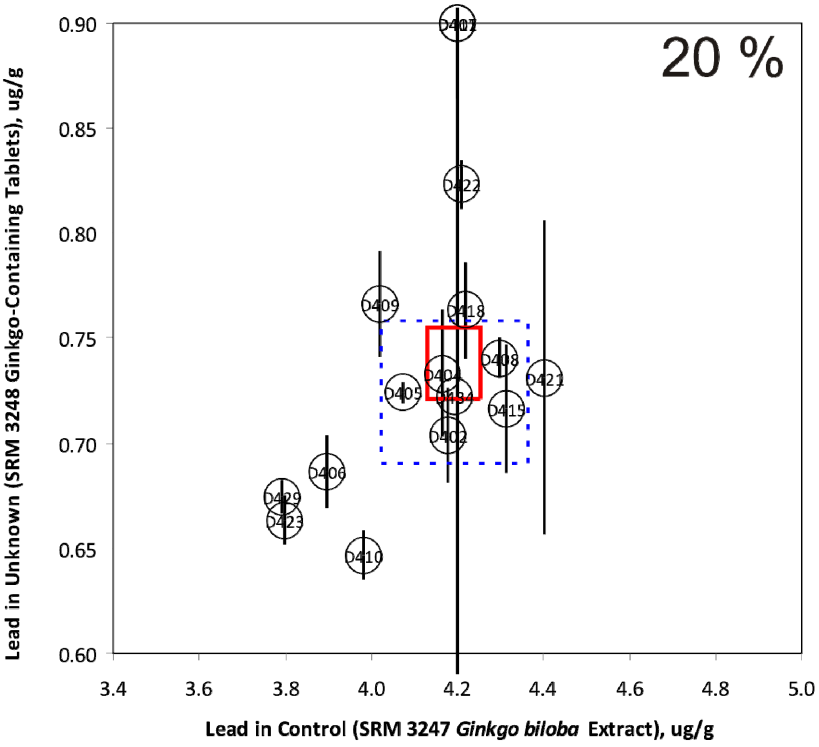
The sample used for the determination of lead was comprised of ground and homogenized ginkgo-containing tablets. The control was SRM 3247 *Ginkgo biloba* extract. The concentration of lead in the control was approximately 5 times higher than the concentration of lead in the sample. The NIST value was adjusted from the certified value to an "as-received" value. There was very good agreement among the results compared on an "as-received" basis. This is discussed further in the recommendation section.

- Twenty-five laboratories enrolled in this exercise and received samples, 17 laboratories reported results (68 %).
- View 1 shows that approximately half of the laboratories fall within the target zone with a roughly equal distribution of high and low results.
- Most laboratories are making very precise measurements as indicated by the relatively small error bars.
- View 2 shows that in many cases the laboratories that reported low values for the control also reported low values for the sample. This trend is typical of either incomplete sample digestion or a calibration error. Because these samples are relatively easy to digest, a calibration error is a possible source of error. A depression of signal can occur in samples read at the high end of the calibration curve giving low values.

Lead View 1



Lead View 2



Return to Introduction

NIACIN AS NIACINAMIDE

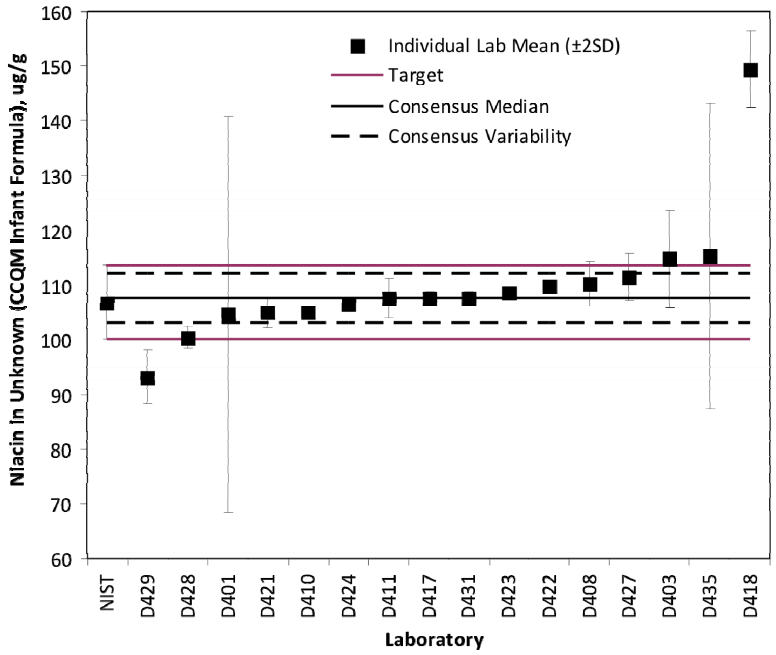
The milk powder used for this exercise was fortified with fat-soluble vitamins, water-soluble vitamins, elements, and fatty acids. The control was SRM 1849 Infant/Adult Nutritional Formula. The level of niacinamide in the control is very similar to the level of niacinamide in the fortified milk powder. This study was a repeat of a study from Exercise C where SRM 1849 was the unknown sample and SRM 3244 Ephedra-Containing Protein Powder was used a control. In Exercise C, the results were extremely scattered with values ranging over two orders of magnitude and approximately half of the results within the target range. In Exercise D, a vial of 500 mg of USP Niacinamide was sent to the participating laboratories for use as a calibrant. A comparison of the results from the two exercises is shown in the recommendation section.

- Twenty-three laboratories enrolled in this exercise and received samples, 16 laboratories reported results (69.5 %).
- The results were significantly improved compared with Exercise C. The consensus values mirrored the target range and only a few laboratories were significant outliers.
- This exercise highlights the importance of calibration materials. When all laboratories used the same calibration material there was good agreement among the data. This indicates that reference materials must be appropriate (niacinamide rather than nicotinic acid) and screened for purity for increased measurement comparability.

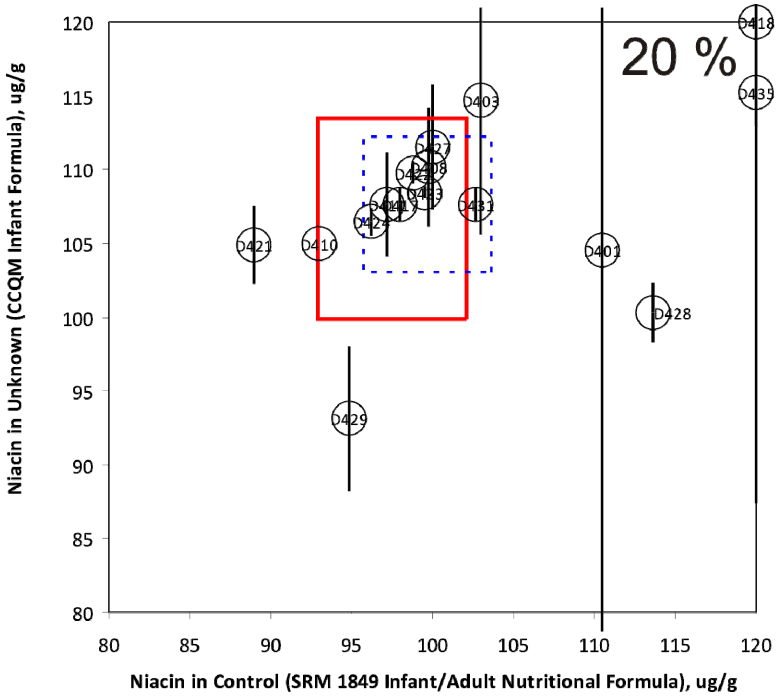
Recommendations:

- A new niacin study in a different matrix (breakfast cereal) without a common calibration material.

Niacin View 1



Niacin View 2



[Return to Introduction]

β-CAROTENE IN OILS

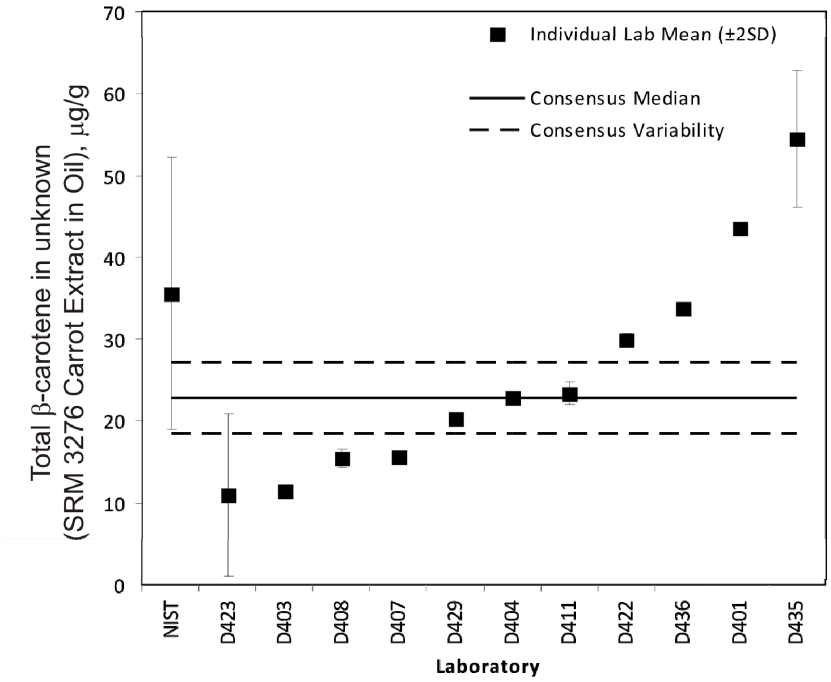
Two botanical extracts in oil were sent for this study. A carrot extract in oil was the sample with SRM 3251 *Serenoa repens* Extract as a control material. The level of β-carotene in the sample was approximately 30 % lower than the level in the control. Both samples required minimal sample preparation as they could be diluted in organic solvent with no additional extraction required.

- Twenty-two laboratories enrolled in this exercise and received samples, 11 laboratories reported results (50 %).
- There is significant scatter in the results, with a low of ~10 μg/g and a high of ~ 55 μg/g total β-carotene.
- Only two laboratories provided specific details about calibrant preparation and only one laboratory provided information on the solvent and molar absorptivity used for the spectrophotometric determination of the calibrant concentration.

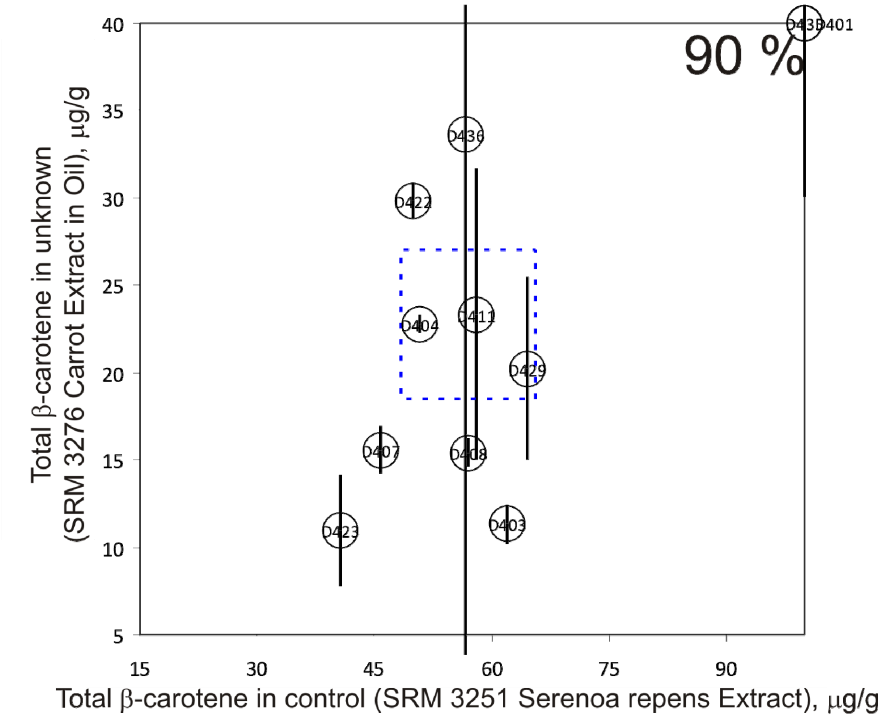
Recommendations:

- Check form of β-carotene reported.
- Check molar absoptivity used for the determination of calibrant concentration. If different laboratories are using different values, there is the potential for wide variation in the results.
- Repeat this study with different samples.

Total β-carotene View 1

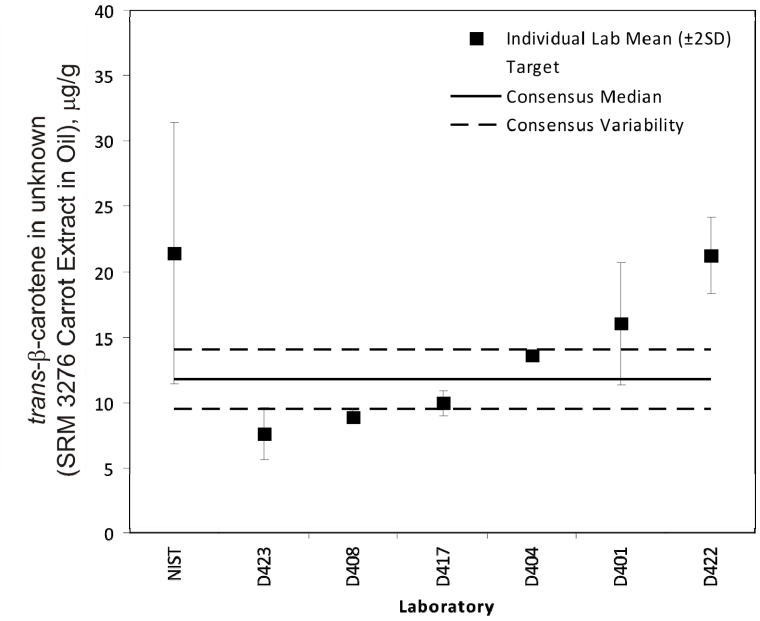


Total β-carotene View 2

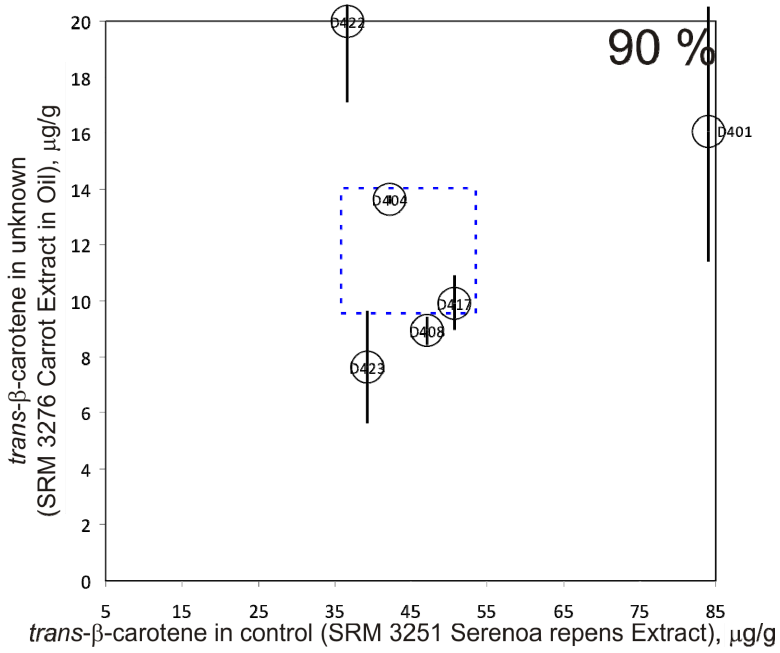


Return to Introduction

trans-β-carotene View 1

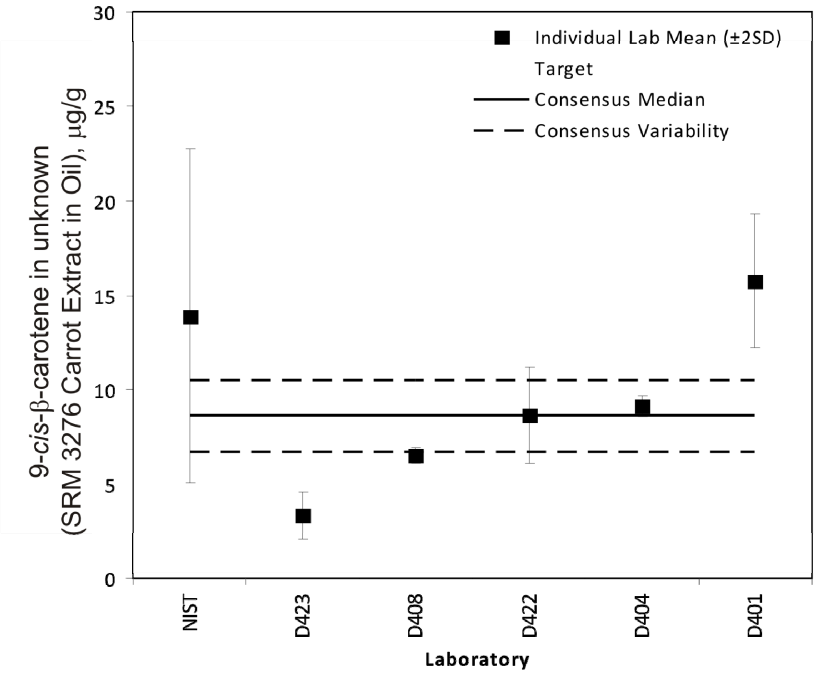


trans-β-carotene View 2

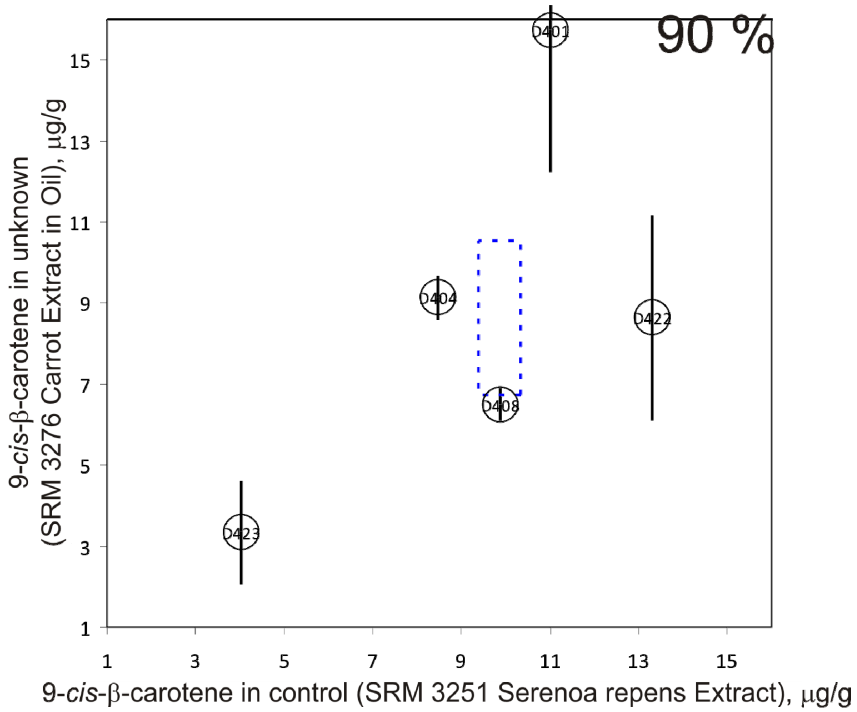


Return to Introduction

cis-β-carotene View 1



cis-β-carotene View 2



[Return to Introduction]

ORGANIC ACIDS IN BERRY PRODUCTS

The berry sample was a spray-dried bilberry extract with SRM 3283 *Vaccinium macrocarpon* (Cranberry) Extract as a control material. The levels of quinic and malic acids in the sample were approximately 30 % lower than the levels in the control material. The level of citric acid is very similar in the sample and the control.

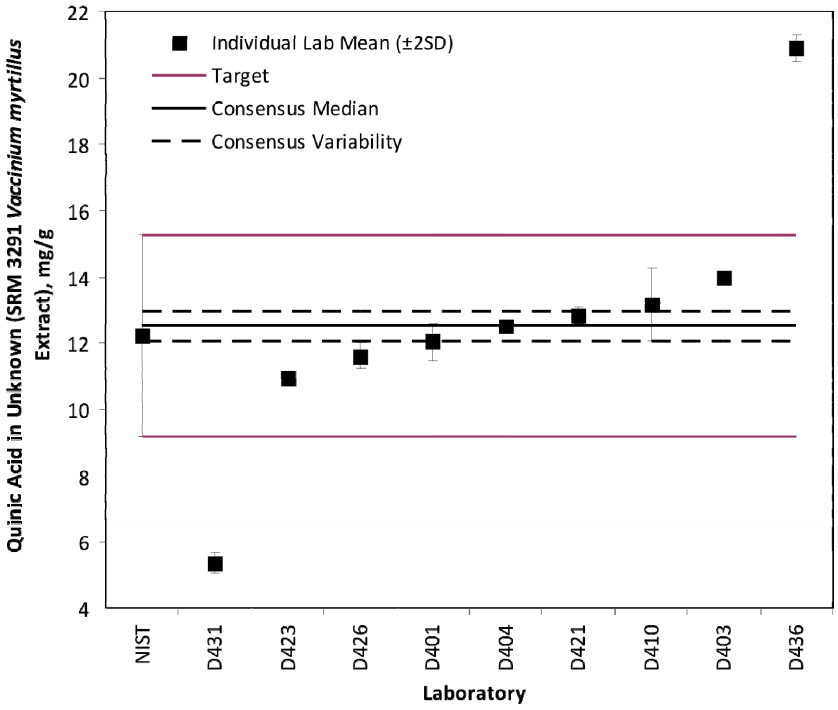
Overall the precision was good for all of the organic acid measurements resulting in tight consensus values. Additionally there was reasonably good agreement with the target values for the unknown sample. Surprisingly there was more variability in the determination of organic acids in the control material than the unknown sample.

- Seventeen laboratories enrolled in this exercise and received samples, 9 laboratories reported results (53 %).
- From information provided by some laboratories, it appears that there were chromatographic interferences in the control that effected results, particularly for quinic acid.
- Laboratories that utilized solid-phase extraction (SPE) for sample cleanup tended to have low results for all of the organic acids.

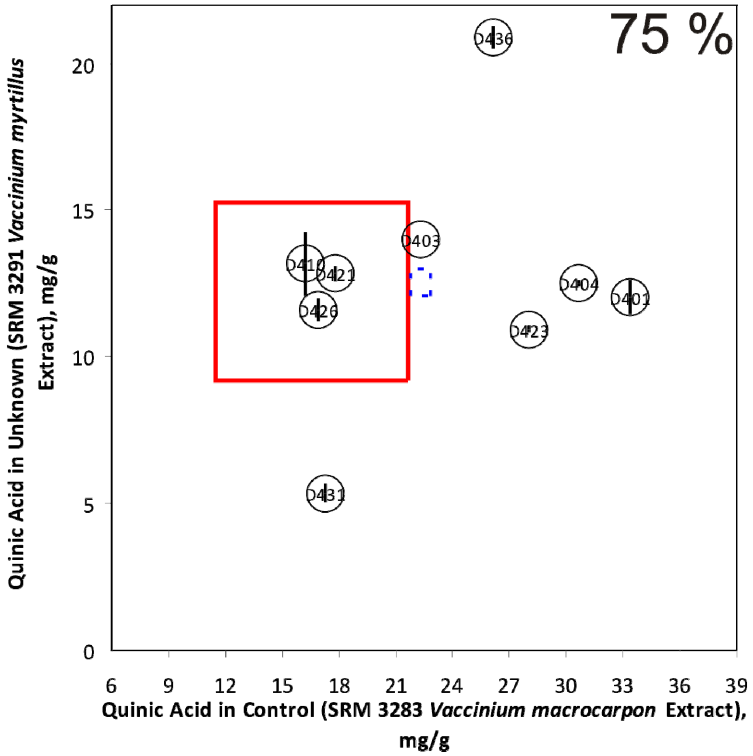
Recommendations:

- Check filters and SPE cartridges with calibrants to determine recovery

Quinic Acid View 1

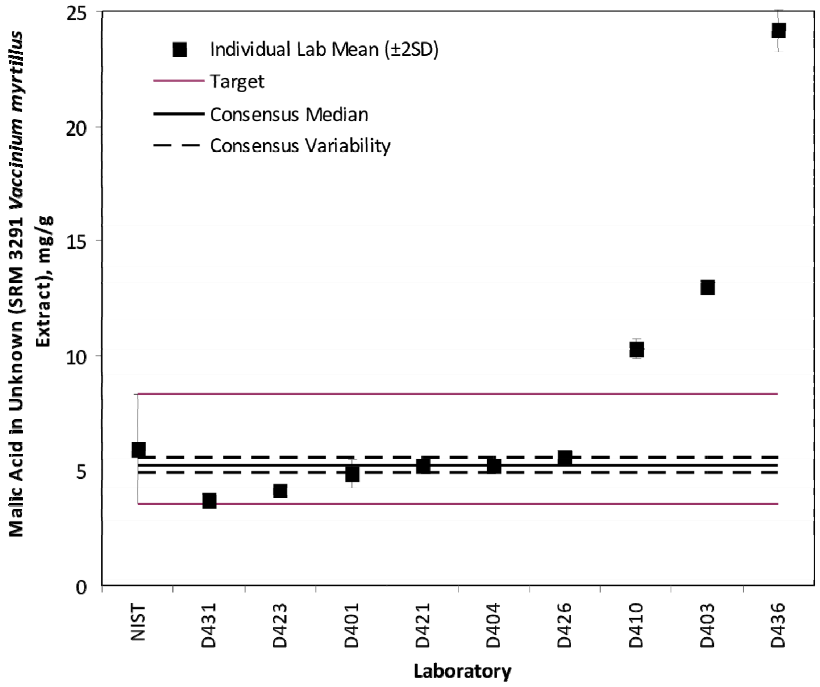


Quinic Acid View 2

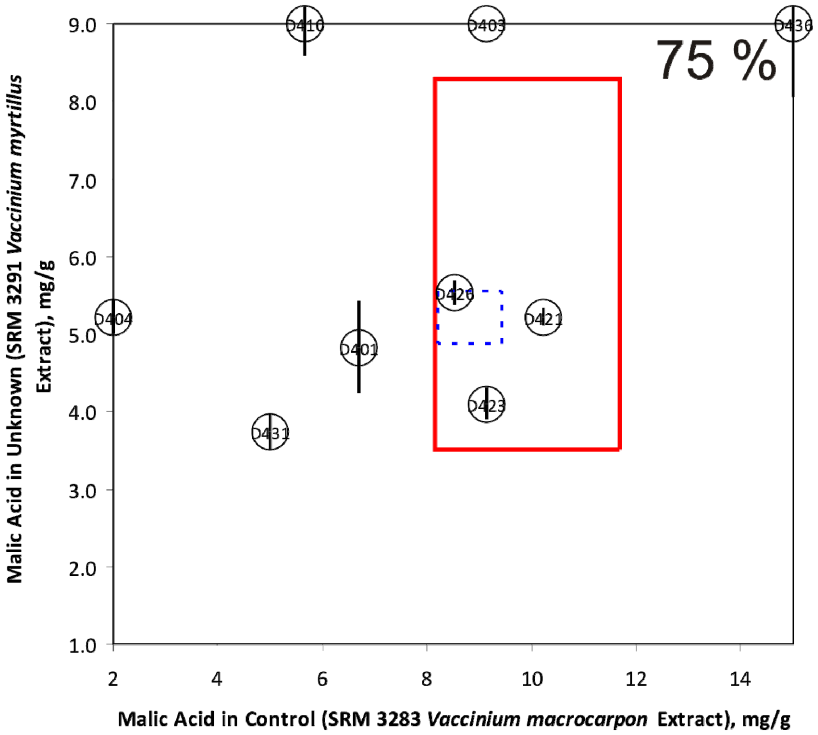


Return to Introduction

Malic Acid View 1

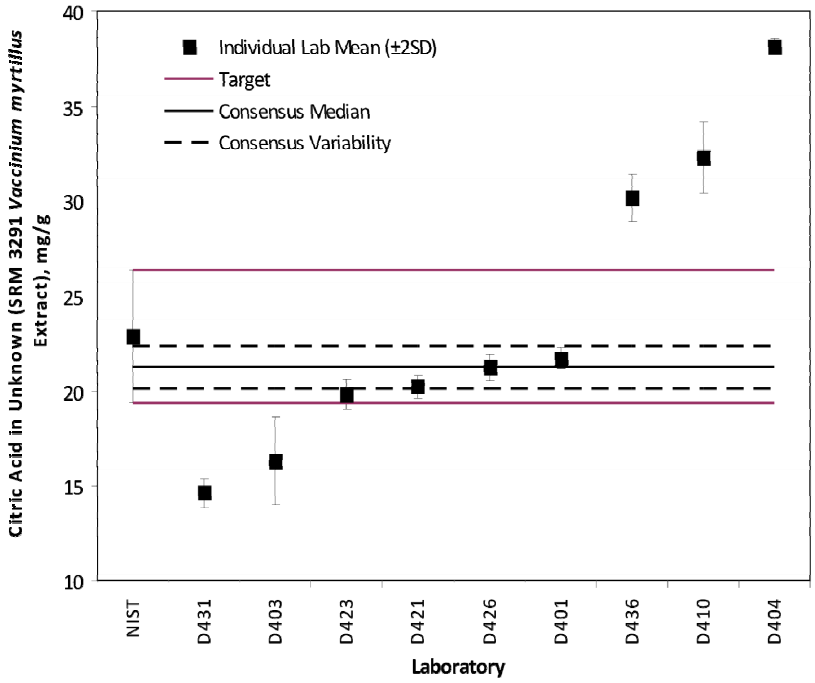


Malic Acid View 2

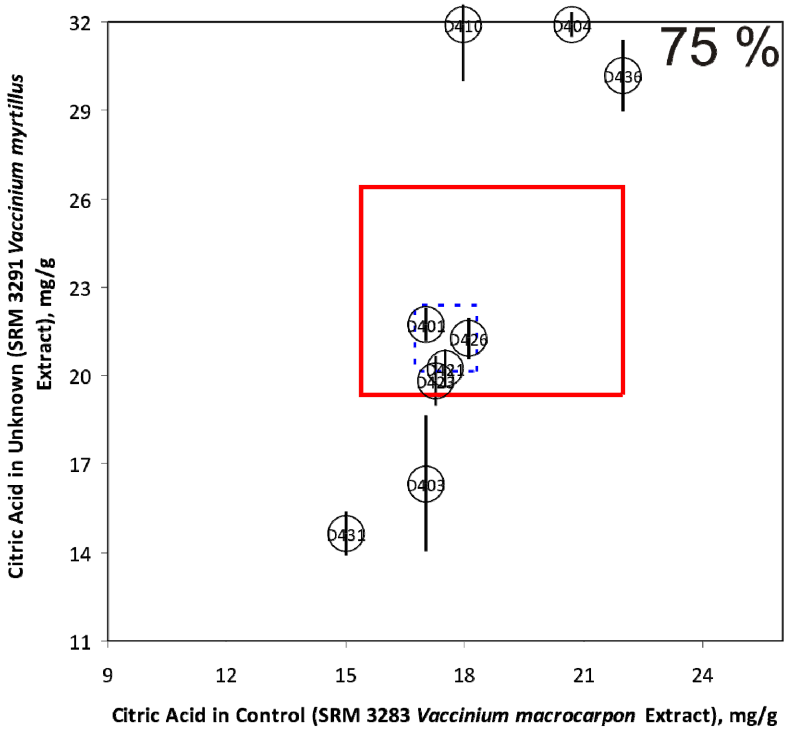


Return to Introduction

Citric Acid View 1



Citric Acid View 2



[Return to Introduction]

RECOMMENDATIONS

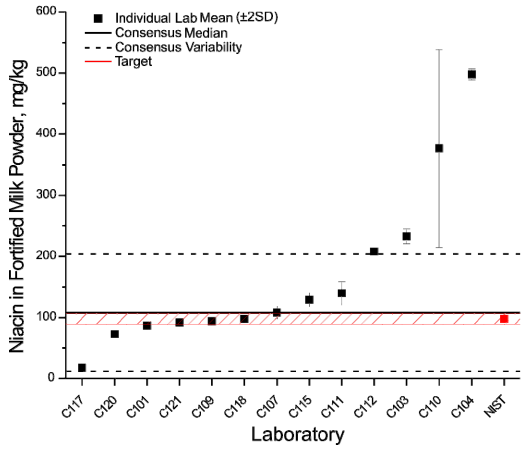
There were several global issues that we noticed while reviewing the data; please consider these recommendations as they may help improve measurement accuracy and precision.

When a target range is provided for a control, the information should be used as a quality self check. The target value should NOT be used to determine a scaling factor. The matrices are often significantly different between the sample and control. Analytes may be extracted differently from the two materials, thus a mathematical adjustment for recovery is not valid.

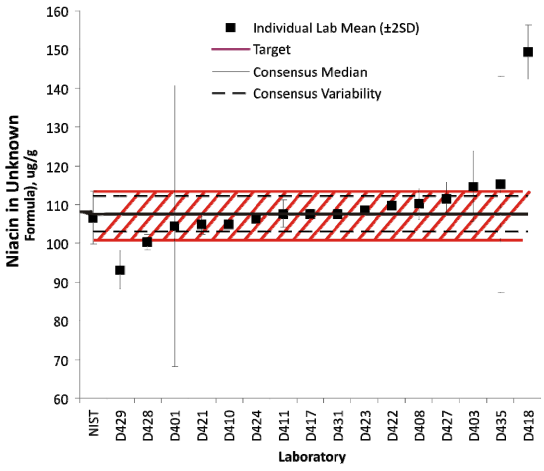
Sample cleanup with solid-phase extraction or filters may be useful prior to analysis. However, the cleanup method should always be tested with a calibrant during method development to ensure that the analytes of interest are not binding to the cleanup substrate.

Evaluation of the purity and stability of calibration materials is very important; this point is highlighted by the measurement of niacin as niacinamide in Exercises C and D. In Exercise C, the results were extremely scattered with values ranging over two orders of magnitude and approximately half of the results within the target range. In Exercise D, a vial of 500 mg of USP Niacinamide was sent to the participating laboratories for use as a calibrant. The results from Exercise D are much improved, with nearly identical target and consensus zones, indicating that the variability in Exercise C is due to differences in calibration materials and not due to the extraction and instrumental methods.

Results from exercise C



Results from Exercise D



When using Standard Reference Materials or other quality control materials it is important to determine whether or not the target range has been adjusted for moisture content, even when the material appears to be a "dry" powder. In Figure A below, laboratories have reported values for lead on an as-received basis and the target range is shown on a dry-mass basis. In Figure B, the target range has been adjusted to an as-received value. The difference is significant!

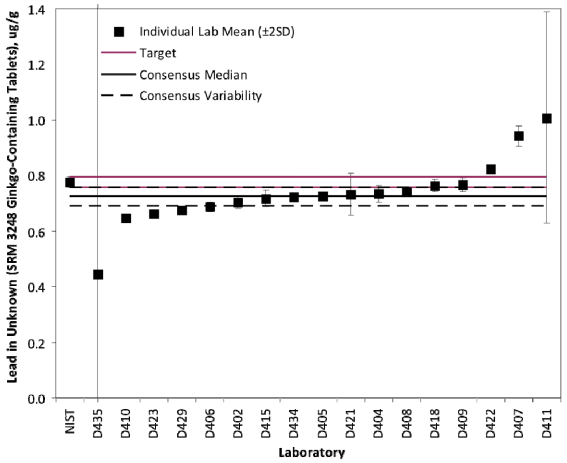


Figure A
Target value reported on dry mass basis, data reported "as received"

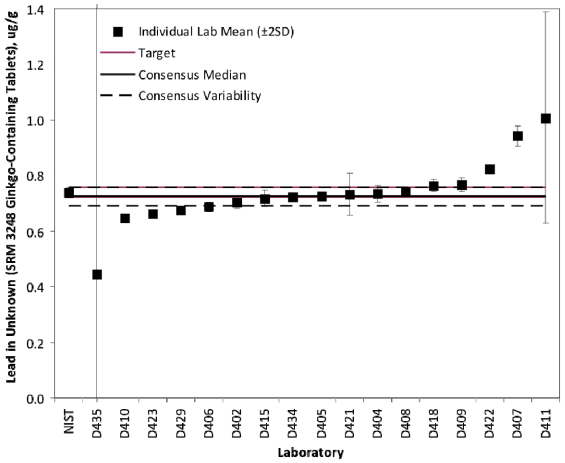


Figure B
Target value and data reported "as received"