Tobacco and Tobacco Products Analytes Sub-Group

Technical Report

2022 Collaborative Study for the Determination of Nitrate and Nitrite in Tobacco and Tobacco Products using Ion Chromatography

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1. Summary

In late 2021, the CORESTA Tobacco and Tobacco Products Analytes Sub-Group (TTPA) initiated an interlaboratory study for the determination of nitrate and nitrite in tobacco and tobacco products by Ion Chromatography (IC). This study included a variety of samples including ground tobacco, cigarette filler, cigar filler and four styles of smokeless tobacco. The intent of this study was to draft a technical report to include repeatability (r) and reproducibility (R) values, z-scores, and a CRM if supported by the study results. The results of the study demonstrate that the method is suitable for the determination of nitrate in tobacco and tobacco products. However, the results for nitrite were inconsistent and suggested either the laboratories needed further practice implementing the method or the method needed additional development for use as an international consensus standardized method.

2. Introduction

In 2016, the CORESTA Smokeless Tobacco Sub-Group (now named Tobacco and Tobacco Products Analytes Sub-Group, TTPA) conducted an investigational interlaboratory study comparing the same IC method used in this collaborative study and the various continuous flow analysis (CFA) methods used by six of the participating laboratories[1]. The intent of this study was to assess the need for an IC method for the determination of nitrate and nitrite. The study results demonstrated that the supplied IC method and the in-house CFA methods were comparable and suitable for the determination of nitrite and nitrate in smokeless tobacco products. However, at that time, the TTPA decided the development of a CRM using IC was not necessary.

In the spring of 2020, the TTPA conducted a survey where 17 laboratories stated an interest in the development of a CRM for nitrate and nitrite using the same IC method provided in the 2016 investigational study[1]. The TTPA initiated a collaborative study where 11 laboratories expressed an interest to participate in the study using the supplied IC method. The protocol for this study was distributed in December 2021 and the study was conducted in December 2021 through February 2022. Data were collected from the 7 laboratories that supplied results and statistically evaluated in basic conformance with the recommendations of ISO 5725-5:1998.

3. Organization

3.1 Participants

A list of the participating laboratories is provided in Table 1. Not all laboratories provided data for all analytes. The laboratories are listed in alphabetical order. Codes were assigned to each laboratory and do not correspond to the order in the table below.

Table 1: List of Participating Laboratories

Participating Laboratories
Altria Client Services LLC, United States
Global Laboratory Services Inc., United States
Imperial Brands, Hamburg, Germany

[1] TTPA Technical Report: Determination of Nitrite and Nitrate in Smokeless Tobacco Products by Ion Chromatography and Continuous Flow Analysis - 2016 Collaborative Study, July 2017 [TTPA-114-CTR]

Participating Laboratories
JTI Ökolab; Vienna, Austria
RJ Reynolds Tobacco Company, United States
Swedish Match North America, United States
University of Kentucky, United States

3.2 Protocol

The study protocol is provided in Appendix A and specific details from the protocol are described below:

3.2.1 Sample Shipment

Laboratories were responsible for procuring the samples from the University of Kentucky CTRP (UKY) and North Carolina State University (NCSU). Laboratories were requested to store the samples at approximately 4 °C if they were analyzed within 1 week. Samples held longer than 1 week prior to analysis were to be placed in moisture barrier bags and stored in the freezer at approximately −20 °C. Laboratories were requested to conduct the study in December 2021 through March 2022 and report data by March 1, 2022. Due to delays and the ongoing pandemic, the study remained open until February 2022. The samples are identified in Table 2.

Table 2: Sample Identification

Sample Name	Description	Supplier
CRP1.1	Swedish-style Snus	NCSU
CRP2.1	American-style loose moist snuff	NCSU
CRP3.1	American-style dry snuff powder	NCSU
CRP4.1	American-style chopped loose-leaf chewing tobacco	NCSU
RT1	1R6F cigarette filler, ground	UKY
RT3	Oriental tobacco, ground	UKY
RT6	Flavoured cigar filler, ground	UKY
RT8	Unflavoured cigar filler, ground	UKY

3.2.2 Within Laboratory Sample Preparation

Participants were directed to transfer the samples from the freezer to the refrigerator a minimum of 24 hours prior to preparation. Next, the samples were to be transferred from the refrigerator to room temperature for a minimum of 2 hours prior to analysis. This procedure ensured there was sufficient time for water to fully re-equilibrate within the products. Once samples are equilibrated to ambient temperature, the samples were to be stored at approximately 4 °C for up to one week if the analyses were not conducted immediately.

The laboratories were directed to follow the instructions given below for each analysis:

- For CRP1.1: Unit portions shall be analyzed together with the pouch material and shall be cut into two halves and added directly into the extraction flask. Add a sufficient number of pouches (2 pouches) to reach the target weight specified in the method.
- All other samples shall be analyzed without grinding. Mix the samples in the container prior to aliquoting.

3.2.3 Sample Analysis and Data Reporting

The participating laboratories were instructed to conduct three (3) independent replicate analyses for each sample. The laboratories were requested to use the supplied test method (Appendix B) and provide data on an as-is basis without correction for moisture.

Participating laboratories were requested to document any deviations from the protocol and submit the deviations with their results. Minor deviations from the supplied IC method are shown below. These results were included in the statistical analysis.

- Lab 2: Calibration standards made from ISO certified stock by Inorganic Ventures, samples shaken at 200 RPM on linear shaker- shaker (speed could not be increased to 250 rpm). Autosampler was at ambient temperature.
- Lab 3: Integration type: peak area is used for both nitrite and nitrate instead of peak height.
- Lab 4: IC - 2 mm System used (IonPac AS19 2 mm x 250 mm with guard column and suppressor-ADRS 600 2 mm).
- Lab 5: System flow rate was set to 0,65 mL/min to operate within safe limits of eluent generator and as such run time (45 min) and eluent concentration gradient was adjusted to reflect the extended run time.

All test results were to be reported on an as-is basis with no correction for moisture content. The results were reported with two significant figures.

The study results and the comments were to be sent by e-mail to Yevgeniya V. Prepelitskaya and Karl Wagner.

4. Data

The full data set for the study is provided in Appendix C. The results are presented on an as-is basis, without correction for moisture. Each analysis includes three replicates. Not all laboratories provided data for all samples or analytes. Raw data plots that include all replicates are given in Appendix D. The results for nitrite were generally variable or not reportable (i.e., below the limit of detection) by several of the participating laboratories. The nitrite results indicate that either the method needs additional refinement to be used by a range of laboratories or the laboratories may need additional practice implementing the method. Due to the limited nitrite data sets, it was not possible to conduct a statistical analysis for nitrite.

5. Statistical Analysis

As mentioned above, the statistical analysis was only conducted for nitrate. The statistical analysis was conducted in basic conformance with ISO 5725-5:1998. This analysis protocol does not identify and remove outliers, rather it uses calculation algorithms that limit the impact of outliers. The calculated results for repeatability (r) and reproducibility (R) are given below in sections 5.1. Even though ISO 5725-5:1998 does not suggest calculation of z-scores, z-scores are presented in section 5.2 so that the participating laboratories would have an additional measure of their performance compared to their peers. Raw data plots that include all replicates are shown in Appendix D.

5.1 Calculation of Repeatability (r) and Reproducibility (R)

The repeatability (r) and reproducibility (R)results are shown in Table 3. The r & R results reflect both laboratory variability and product consistency. It should be noted that the number of labs is small, so this estimated variability is less reliable than would be the case with more participants. Even considering the relatively small number of participating laboratories, the estimated reproducibility for nitrate compares well with other tobacco analyses. For reference, the Horwitz-Thompson estimated reproducibility estimates are also shown in the table and these results vary from comparable to the Horwitz-Thompson estimated values to much better.

Table 3: Repeatability (r) and Reproducibility (R) Results for Nitrate

Sample	N° of Labs[1]	Nitrate Mean	Repeatability[2]		Reproducibility[2]		HT Predicted R[3]
			r	% r	R	% R	
CRP1.1	7	5894	298	5,1 %	719	12,2 %	12,1 %
CRP2.1	7	17158	299	1,7 %	1411	8,2 %	10,3 %
CRP3.1	7	38371	2733	7,1 %	2733	7,1 %	9,1 %
CRP4.1	7	7558	81,6	1,1 %	716	9,5 %	11,7 %
RT1	7	7046	146	2,1 %	255	3,6 %	11,8 %
RT3	7	483	30,8	6,4 %	61,5	12,7 %	17,7 %
RT6	7	18140	186	1,0 %	1119	6,2 %	10,2 %
RT8	6	9021	138	1,5 %	497	5,5 %	11,4 %

1. The number of laboratory data sets included in the r & R calculations.
2. % r and % R reflect the corresponding variability value divided by the mean and expressed as a percent.
3. HT Predicted R is based on the Horwitz-Thompson equation.

5.2 Calculation of Z-Scores

Although calculation of z-scores was not integral for the objectives of the study, z-scores were calculated so that the participating laboratories could compare their results to those of their peers and aid them in maintaining laboratory accreditation.

The z-scores were calculated using methods suggested in ISO 13528: 2015. The formula for the calculation is $z_i = \frac{x_i - \bar{x}}{\sigma}$ where x_i is the lab average for Lab i for the given product and analyte, $\bar{x}$ and σ are the corresponding product mean and standard deviation for the analyte given in Table 4 and calculated using Algorithm A as described in ISO 13528:2015.

It is expected that most of the data should fall within the range of ±2, and that laboratories having values with |z| > 3 should be treated as an "action signal" to investigate laboratory performance. Final summary tables of z-scores are presented in the table below. However, it should be borne in mind that if the standard deviations are small, relatively small deviations can result in large z-scores. For example, the largest absolute z-score in the table is Lab 6 for RT1. However, the value is less than 4 % from the mean, so should not be judged too negatively. This large z-score appears to be caused by a very small estimated standard deviation, not by an excessively large deviation from the average. The z-scores are shown graphically in Appendix E.

Table 4: Z-Scores for Nitrate

Lab	CRP1.1	CRP2.1	CRP3.1	CRP4.1	RT1	RT3	RT6	RT8
Mean	5894	17158	38371	7558	7046	483	18140	9021
StDev	242	496	471	254	80,6	20,0	396	173
1	-1,19	-0,09	-0,32	0,30	-0,52	-0,59	-0,37	-0,01
2	0,45	-0,23	-0,42	0,11	-0,50	-0,75	-0,65	0,05
3	-0,45	0,45	0,90	-0,72	0,55	0,83	0,04	-0,02
4	2,21	-1,04	-0,72	1,29	1,05	0,00	1,12	–
5	0,25	1,39	-0,90	0,26	0,26	-0,09	0,35	0,22
6	-0,71	-1,03	-0,05	-1,52	-3,30	-0,90	-1,39	-1,80
7	0,16	0,55	1,59	0,26	0,65	1,54	0,90	1,26

The (–) symbol indicates the laboratory did not submit data for that sample analysis.

6. Data Interpretations

There were not as many participating laboratories as would be preferred, but, nonetheless, the nitrate results were generally consistent between the laboratories. The method is fit for purpose for the analysis of nitrate. However, the results for nitrite were more variable or not reportable (i.e., below the limit of detection). The nitrite results indicate that either the method needs additional refinement to be used by a range of laboratories or the laboratories may need additional practice implementing the method.

7. Recommendations

The results of this study demonstrate that the supplied IC method is suitable for the analysis of nitrate in tobacco and tobacco products, and it is therefore recommended to draft a CRM using IC for nitrate.

APPENDIX A: Protocol

CORESTA TOBACCO and TOBACCO PRODUCTS ANALYTES SUB-GROUP

Project Title: Determination of Nitrate and Nitrite in Tobacco and Tobacco Products using Ion Chromatography

Type of Document: Collaborative Study Protocol

Date: November 30, 2021

Written by: Genya Prepelitskaya, Tommy Hurst

Confidentiality Notice: All data submitted by participating laboratories will be coded and kept confidential

1. Introduction

At the virtual CORESTA Tobacco and Tobacco Products Analytes Subgroup (TTPA) meeting held on October 14, 2021, the group decided to conduct a collaborative study for nitrate and nitrite in tobacco and tobacco products prior to the spring 2022 meeting.

2. Objective

The objective of this study is to develop a CORESTA Recommended Method (CRM) for the determination of nitrate and nitrite in tobacco and tobacco products using ion chromatography. This study will include a variety of samples including tobacco, cigarette filler, cigar filler and a variety of styles of smokeless tobacco. The final output will be a technical report that will include repeatability (r) and reproducibility (R) values, z-scores, and a CRM. The results will be presented at the spring TTPA meeting.

3. Time schedule

Table 1: Study timeline

Date	Activity
December 2021 – March 1, 2022	Distribute the study protocol and data reporting sheet and laboratories conduct the study
	Additional Laboratories state their intention to participate
March 1, 2022	Laboratories submit results by this date
TBD, 2022	Discuss results at Spring 2022 TTPA meeting

4. Participating Laboratories

The laboratories listed in Table 2 have kindly agreed to take part in the study. Other laboratories are encouraged to participate and should notify Genya Prepelitskaya, Tommy Hurst, and Karl Wagner of their interest to participate.

Table 2: Participating Laboratories

Participating Laboratories
Altria Client Services LLC, United States
Swedish Match, Owensborro, United States
Global Laboratory Services LLC, United States
University of Kentucky, United States
Enthalpy Analytical, LLC, Richmond, United States
ITC Limited, India
JTI Okolab, Austria
Swisher International, Inc., United States
R.J. Reynolds Tobacco Company, United States
ITG Brands United States
Imperial Tobacco Reemtsma Cigarettenfabriken GmbH, Hamburg, Germany

5. Samples

Participants should order the samples shown in Table 3.

Table 3: Samples

Sample Name	Description	Supplier	Quantity to Order
CRP1.1	Swedish-style Snus	NCSU	3 cans
CRP2.1	American-style loose moist snuff	NCSU	3 cans
CRP3.1	American-style dry snuff powder	NCSU	3 cans
CRP4.1	American-style chopped loose-leaf chewing tobacco	NCSU	3 cans
RT1	1R6F filler, ground	UKY	2 bottles
RT3	Oriental tobacco, ground	UKY	2 bottles
RT6	Flavoured Cigar Filler, ground	UKY	2 bottles
RT8	Unflavoured Cigar Filler, ground	UKY	2 bottles

- Note: The 2016 CRPs must be ordered from North Carolina State University: https://strp.wordpress.ncsu.edu/ordering/. Please contact Karen Andres (karen_andres@ncsu.edu) with questions regarding ordering and shipping.
- Note: RT1, RT3, RT6, and RT8 samples must be ordered from the University of Kentucky: https://ctrp.uky.edu/. Do not use 1R6F filler that has been removed from cigarettes.

6. Sample Storage

Unless analyzed within one week, all samples should be placed in moisture barrier bags and stored at −20 °C prior to analysis.

If you choose to use samples from in-house inventory, ensure they have been stored unopened and at the recommended long term storage conditions of -20 °C. CRP samples previously opened shall not be used for this study.

7. Analysis

7.1 Analytes:

Report nitrate and nitrite in each sample in the data reporting sheet provided. Report data on an as-is basis.

7.2 Methods:

The supplied test method must be used for the analysis. Since the purpose of this study is to develop a CRM, data generated with methods other than the one supplied will not be included in this study.

7.3 Replicates:

Conduct three (3) independent replicate analyses for each sample. The replicates should be determined from independent tobacco aliquots from the same bottle or can.

7.4 Sample equilibration:

Samples held at -20 °C shall be placed unopened in a refrigerator for a minimum of 24 hours to ensure water has fully equilibrated within the product. Samples shall be removed from the refrigerator a minimum of 2 hours prior to opening for analysis. The samples shall not be opened during the time the samples are equilibrating to ambient temperature. Once samples are equilibrated to ambient temperature, the samples may be stored at approximately 4 °C for up to one week if the analyses will not be conducted immediately.

7.5 Sample Handling Requirements:

- CRP1.1: Unit portions shall be analysed together with the pouch material and shall be cut into two halves directly into the extraction flask. Add a sufficient number of pouches (2 pouches) to reach the target weight specific in the method.
- All other samples shall be used without further grinding. Mix the samples in the container prior to aliquoting.

7.6 Data Reporting:

- Input the final data into the provided data report spreadsheet.
- Report nitrite to 2 decimal places and report nitrate to the single digit.
- List any deviations from the supplied test method in the data reporting sheet.
- Forward the completed data reporting sheet to the study coordinators Genya Prepelitskaya, Tommy Hurst, and Karl Wagner.

8. Statistical Analysis

A statistical analysis in general conformance with ISO 5725-2:1994 and ISO/TR 22971:2005 will be conducted. Repeatability (r) and reproducibility (R) values will be reported as will z-scores.

9. Presentation of the Results

The results will be presented for discussion at the Spring 2022 TTPA meeting.

APPENDIX B: Draft CORESTA Recommended Method

Determination of Nitrite and Nitrate in Tobacco and Tobacco Products by Ion Chromatography

November 30, 2021

0. INTRODUCTION

To be written after the study

1. FIELD OF APPLICATION

This method is used to quantitatively determine the concentration of nitrite and nitrate in tobacco, cigarette filler, moist smokeless tobacco (MST) and snus using ion chromatography. Results are reported in units of µg/g on an as-is basis.

This method is applicable to samples with concentrations of nitrite in the range of 2 - 750 µg/g and nitrate in the range of 50 - 20000 µg/g.

2. NORMATIVE REFERENCES

CORESTA Guide N° 11 - Technical Guideline for Sample Handling of Smokeless Tobacco and Smokeless Tobacco Products

ISO 3696, Water for analytical laboratory use – Specification and test methods

3. TERMS AND DEFINITIONS

No terms and definitions are listed in this document.

4. PRINCIPLE

The nitrite and nitrate in tobacco and tobacco products is determined by extracting a tobacco sample in Type 1 water. The extract is then analyzed by ion chromatography using suppressed conductivity detection. The results are reported as micrograms of analyte per gram of tobacco.

5. APPARATUS

0.1. Ion Chromatograph (IC) consisting of an eluent generator, conductivity detector, conductivity suppressor, temperature controlled autosampler, and data collection system. An eluent degassing unit is recommended.

0.2. Weak anion exchange column of mid-capacity, (4 mm x 250 mm, nonmetallic), approximately 240 µeq per column with matching guard column[2].

[2] ThermoFisher IonPac® AS19 anion exchange analytical column is the trade name of a suitable product available commercially. Other column(s) may be suitable for use with this method; however, laboratories must verify suitable resolution in the test samples before use. This information is given for the convenience of the users of this Recommended Method and does not constitute endorsement of this product.

0.3. Balance, 3-place, 0,001 g precision.

0.4. Orbital platform shaker with square platform

0.5. Containers for sample extraction: 125-ml glass Erlenmeyer flask or similar flask of glass or plastic for extracting samples

0.6. 1000 ml class A volumetric flask

0.7. 100 ml class A volumetric flask

0.8. 0,2 µm PVDF syringe filter, 25mm

6. REAGENTS

0.9. Sodium nitrite ($NaNO_2$) > 99 % purity

0.10. Sodium nitrate ($NaNO_3$) > 99 % purity

Note: Certified reference standards of 100 µl/ml nitrite and 1000 µl/ml nitrate may also be used

0.11. Type 1 water

7. PREPARATION OF SOLUTIONS

0.12. Stock Solutions:

0.12.1. Nitrite Stock Solution: Weigh 0,15 g of sodium nitrite and record the mass to the nearest 0,001 g. Add the reagent to a 1000 ml volumetric flask and dissolve in Type 1 water. This solution contains approximately 100 µg/ml nitrite. Calculate the exact concentration. Store the prepared solution in a tightly sealed polypropylene bottle in the refrigerator.

0.12.2. Nitrate Stock Solution: Weigh 1.37 g of sodium nitrate and record the mass to the nearest 0,001 g. Add the reagent to a 1000 ml volumetric flask and dissolve in Type 1 water. This solution contains approximately 1000 µg/ml nitrate. Calculate the exact concentration. Store the prepared solution in a tightly sealed polypropylene bottle in the refrigerator.

0.13. Calibration Standard Solutions:

Prepare a series of at least six calibration standard solutions whose concentrations cover the range expected to be found in the test portion. An example calibration range is given in Table. Dilute to volume using Type 1 water.

Table 1. Calibration Standards

	Volume (ml)	Volume Nitrite Stock Solution (ml)	Volume Nitrate Stock Solution (ml)	Nitrite Concentration (µg/ml)	Nitrate Concentration (µg/ml)
Cal 1	100	0,0400	0,100	0,0400	1,00
Cal 2	100	0,200	0,500	0,200	5,00
Cal 3	100	1,00	2,00	1,00	20,0
Cal 4	100	5,00	10,0	5,00	100
Cal 5	100	10,0	25,0	10,0	250
Cal 6	100	15,0	40,0	15,0	400

8. SAMPLE PREPARATION

0.14. Sample Requirements:

0.14.1. Sampling is conducted such that the laboratory test sample is representative of the population to be tested.

0.14.2. A homogeneous test portion shall be prepared for each test sample.

0.14.3. At least 2 grams of tobacco sample per replicate is required for this test method.

0.14.4. Tobacco samples stored in the freezer shall be allowed to equilibrate, unopened, in the refrigerator for a minimum of 24 hours. After equilibration in the refrigerator, samples shall be allowed to equilibrate to ambient conditions before being opened for sample preparation.

Note: Insufficient equilibration time for samples removed from the freezer has been identified as a source of variability. Samples removed from the freezer should be placed unopened in the refrigerator for a minimum of 24 hours to ensure water has sufficient time to fully equilibrate throughout the sample. At the time of analysis, samples should be allowed to equilibrate to room temperature before being opened for weighing.

0.15. Sample Grinding:

Tobacco and tobacco products shall be ground unless the samples are homogeneous and have a particle size <4 mm. It is important that the grinding procedure does not generate excessive heat or cause sample degradation. For further information, see CORESTA Guide no. 11.

0.15.1. Smokeless tobacco products supplied in the form of pouches shall be analyzed together with the pouch material and shall be cut into two halves directly into the extraction flask.

0.15.2. Cigarette filler typically does not need to be ground prior to analysis. Remove the filler from the cigarette paper and filter from a sufficient number of cigarettes to create a representative test sample.

0.15.3. Cigar filler typically needs to be ground prior to analysis. Testing may also involve the analysis of the entire cigar where the wrapper and filler are ground together. However, non-tobacco components such as filters must be removed prior to grinding. Grind a sufficient number of cigars to create a representative test sample.

0.16. Sample Extraction:

0.16.1. Mix the tobacco sample before aliquoting.

0.16.2. Weigh 2,0 g ± 0,3 g of tobacco material with an analytical balance into a tared 125 ml Erlenmeyer flask (or similar). Record the exact weight of sample to the nearest 0,001 g.

0.16.3. When analyzing pouched products, select a unit number of pouches that comes closest to the target weight of 2,0 g. The pouch(s) shall be cut in half and the tobacco and pouch material shall be added to the extraction vessel. Record the exact weight of sample to the nearest 0,001 g.

0.16.4. Add 100,0 ml Type 1 water.

0.16.5. Cover the flask with a lid or equivalent.

0.16.6. Shake samples on an orbital platform shaker or equivalent device at 250 rpm for at least 30 ± 5 minutes. Ensure the shaking speed is sufficient to vigorously agitate the tobacco and for portioned smokeless products, the tobacco should become separated from the pouch material.

0.16.7. Filter approximately 1 ml of sample directly into 1,5 ml autosampler vial using a 0,2 µm PVDF syringe filter.

0.17. Sample Stability:

0.17.1. Sample extracts should be stored in a refrigerator and, ideally, analyzed on an instrument equipped with a temperature controlled autosampler.

0.17.2. Nitrite has been shown to be stable in prepared test samples for up to 48 hours at ambient condition and for up to 7 days when extracts were stored in a refrigerator.

0.17.3. Nitrate has been shown to be stable in prepared test samples for up 7 days at ambient and refrigerated temperatures.

9. SAMPLE ANALYSIS

0.18. Instrument Setup:

The determination of nitrite and nitrate is achieved using suppressed conductivity detection and a potassium hydroxide eluent generator in the recycled mode.

The instrument conditions specified below are recommendations and may be adjusted to obtain suitable chromatography. Set up the apparatus and operate the ion chromatograph in accordance with the manufacturer's instructions. Ensure that the analyte peaks and other tobacco component peaks are well resolved.

Instrument operating conditions that have been found to be suitable for the specified column are as follows:

- Flow rate: 1,0 ml/min
- Column temperature: 30 °C
- Detector cell temperature: 35 °C
- Injection volume: 10 µl
- Suppressor current: 137 mA
- Flush volume: 500 µl
- Calibration type: linear with 1/X weighing and the y-intercept is not forced through zero
- Integration type: peak height is used for nitrite and peak area is used for nitrate
- Run time: 30 min

The gradient profile for eluent generator is listed in the table below:

Table 2. IC Gradient Profile

Time (min)	Concentration of KOH (mM)	Eluent Generator Curve	Flow rate (ml/min)
0	10	5	1,0
12	10	5	1,0
25	55	5	1,0
26	10	5	1,0
30	10	5	1,0

Example chromatograms are provided in the Appendix.

0.19. Calibration:

Inject a 10 µl aliquot of each of the calibration solutions into the ion chromatograph. Generate a linear calibration curve with 1/X weighting without forcing regression through the origin.

Note: it is recommended to verify the calibration by injecting an aliquot of an intermediate concentration standard, which should be prepared from a separate stock, after calibration and after approximately every 20 test portions.

0.20. Determination of the concentration of nitrite and nitrate in the test samples:

Inject a 10 µl aliquot of the Type 1 water used for sample extraction to evaluate for carryover or contamination. Inject 10 µl aliquots of each test portion into the ion chromatograph. Calculate the concentration of the analytes using the calibration curve.

0.21. Expression of results:

The concentration of nitrite or nitrate (in µg/ml) in the test portion is determined by the external standard calibration method using the regression equation derived from the calibration curve.

Results are then converted and reported on a weight/weight basis using the formula below

$$Analyte = \frac{c \times v}{w}$$

Where:

Analyte = the concentration of nitrate or nitrite, on an as-is basis (µg/g)
c = the concentration of nitrate or nitrite from the calibration curve (µg/ml)
v = the volume of the extraction solution, including any dilutions (ml)
W = the weight of the tobacco sample (g)

10. REPEATABILITY AND REPRODUCIBILITY

To be written after the collaborative study (citation [1])

11. TEST REPORT

The test report shall state the amount of nitrite and nitrate in micrograms per gram tobacco (wet weight) and shall include all conditions not specified in this Recommended Method which may affect the results. The report shall also give all details necessary for the identification of each sample. Moisture content may be determined on separate tobacco aliquots if it is necessary to present the final results on a dry-weight basis. The determination of moisture is detailed in CORESTA Recommended Method N˚ 76: Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products [2].

12. BIBLIOGRAPHY

[1] CORESTA Tobacco and Tobacco Products Analytes Sub-Group Technical Report: Determination of Nitrate and Nitrite in Tobacco and Tobacco Products using Ion Chromatography, Month 2022. To be written after the study

[2] CORESTA Recommended Method No. 76: Determination of Moisture Content (Oven Volatiles) of Tobacco and Tobacco Products.

APPENDIX C: Raw Data Set

Full Data Set for Study (results are presented on an as-is basis)

Lab Code	Product	Replicate	Nitrite (µg/g)	LOQ Flag	Nitrate (µg/g)
1	CRP1.1	1	–	<0.67	5526
1	CRP1.1	2	–	<0.67	5784
1	CRP1.1	3	–	<0.67	5509
1	CRP2.1	1	4,02	Above LOQ	17104
1	CRP2.1	2	3,90	Above LOQ	17135
1	CRP2.1	3	3,89	Above LOQ	17102
1	CRP3.1	1	7,85	Above LOQ	38441
1	CRP3.1	2	7,73	Above LOQ	38178
1	CRP3.1	3	7,76	Above LOQ	38048
1	CRP4.1	1	–	<0,67	7659
1	CRP4.1	2	–	<0,67	7622
1	CRP4.1	3	–	<0,67	7620
1	RT1	1	–	<0,67	6990
1	RT1	2	–	<0,67	7052
1	RT1	3	–	<0,67	6972
1	RT3	1	–	<0,67	470
1	RT3	2	–	<0,67	472
1	RT3	3	–	<0,67	473
1	RT6	1	–	<0,67	17908
1	RT6	2	–	<0,67	17950
1	RT6	3	–	<0,67	18118
1	RT8	1	–	<0,67	8977
1	RT8	2	–	<0,67	9045
1	RT8	3	2,01	Above LOQ	9036
2	CRP1.1	1	–	<0,26	6110
2	CRP1.1	2	–	<0,26	5940
2	CRP1.1	3	–	<0,26	5955
2	CRP2.1	1	4,52	Above LOQ	17037
2	CRP2.1	2	4,66	Above LOQ	17040
2	CRP2.1	3	4,62	Above LOQ	17050
2	CRP3.1	1	7,15	Above LOQ	36703
2	CRP3.1	2	9,30	Above LOQ	38896
2	CRP3.1	3	7,33	Above LOQ	38923
2	CRP4.1	1	–	<0,26	7585
2	CRP4.1	2	–	<0,26	7568
2	CRP4.1	3	–	<0,26	7604
2	RT1	1	–	<0,26	6998
2	RT1	2	–	<0,26	7009
2	RT1	3	–	<0,26	7012
2	RT3	1	–	<0,26	468
2	RT3	2	–	<0,26	470

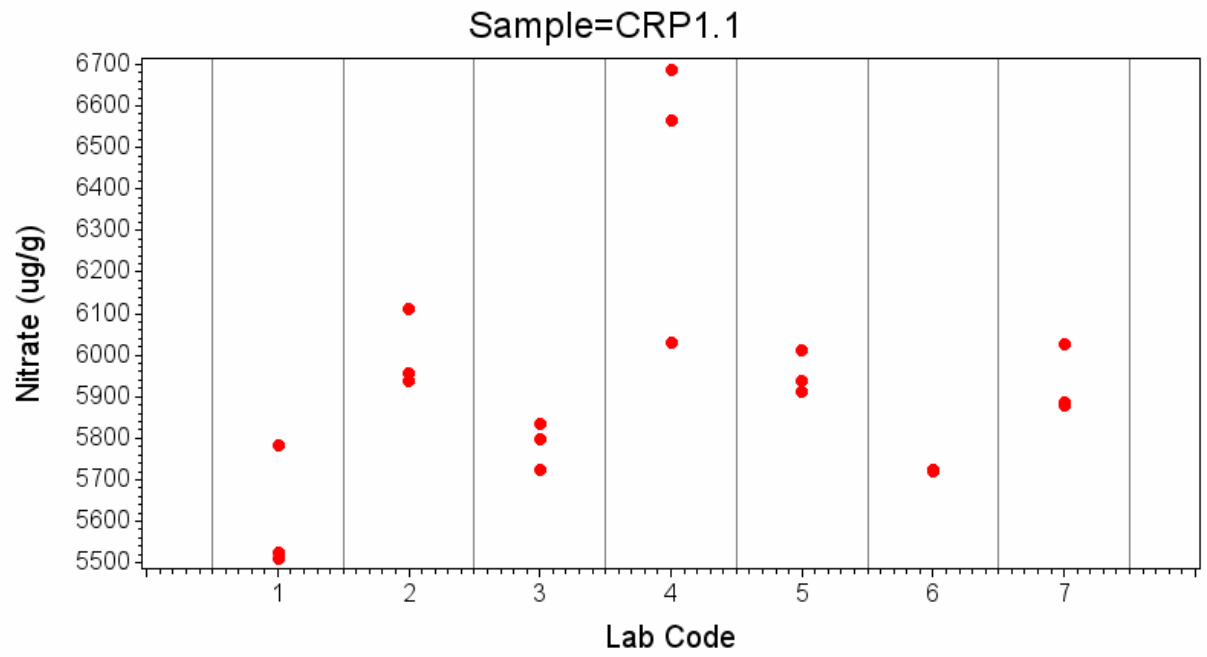
Lab Code	Product	Replicate	Nitrite (µg/g)	LOQ Flag	Nitrate (µg/g)
2	RT3	3	–	<0,26	467
2	RT6	1	2,70	Above LOQ	17872
2	RT6	2	2,56	Above LOQ	17931
2	RT6	3	2,49	Above LOQ	17851
2	RT8	1	2,91	Above LOQ	9034
2	RT8	2	2,89	Above LOQ	9040
2	RT8	3	2,84	Above LOQ	9014
3	CRP1.1	1	–	<LOQ	5834
3	CRP1.1	2	–	<LOQ	5797
3	CRP1.1	3	–	<LOQ	5724
3	CRP2.1	1	3,12	Above LOQ	17536
3	CRP2.1	2	3,02	Above LOQ	17243
3	CRP2.1	3	2,95	Above LOQ	17369
3	CRP3.1	1	12,59	Above LOQ	37225
3	CRP3.1	2	12,40	Above LOQ	39607
3	CRP3.1	3	12,59	Above LOQ	39555
3	CRP4.1	1	3,09	Above LOQ	7465
3	CRP4.1	2	3,09	Above LOQ	7323
3	CRP4.1	3	3,06	Above LOQ	7336
3	RT1	1	2,41	Above LOQ	7140
3	RT1	2	2,30	Above LOQ	7105
3	RT1	3	2,47	Above LOQ	7027
3	RT3	1	1,75	<LOQ	516
3	RT3	2	1,88	<LOQ	490
3	RT3	3	1,88	<LOQ	494
3	RT6	1	3,91	Above LOQ	18193
3	RT6	2	3,61	Above LOQ	18140
3	RT6	3	3,82	Above LOQ	18140
3	RT8	1	4,86	Above LOQ	9019
3	RT8	2	4,63	Above LOQ	9143
3	RT8	3	4,93	Above LOQ	8891
4	CRP1.1	1	–	<0,6	6567
4	CRP1.1	2	–	<0,6	6688
4	CRP1.1	3	–	<0,6	6029
4	CRP2.1	1	3,13	Above LOQ	16772
4	CRP2.1	2	3,04	Above LOQ	16656
4	CRP2.1	3	3,40	Above LOQ	16491
4	CRP3.1	1	9,91	Above LOQ	38052
4	CRP3.1	2	10,71	Above LOQ	38077
4	CRP3.1	3	10,57	Above LOQ	37966
4	CRP4.1	1	–	<0,6	7883
4	CRP4.1	2	–	<0,6	7877
4	CRP4.1	3	–	<0,6	7897
4	RT1	1	1,33	Above LOQ	7151
4	RT1	2	–	<0,6	7166

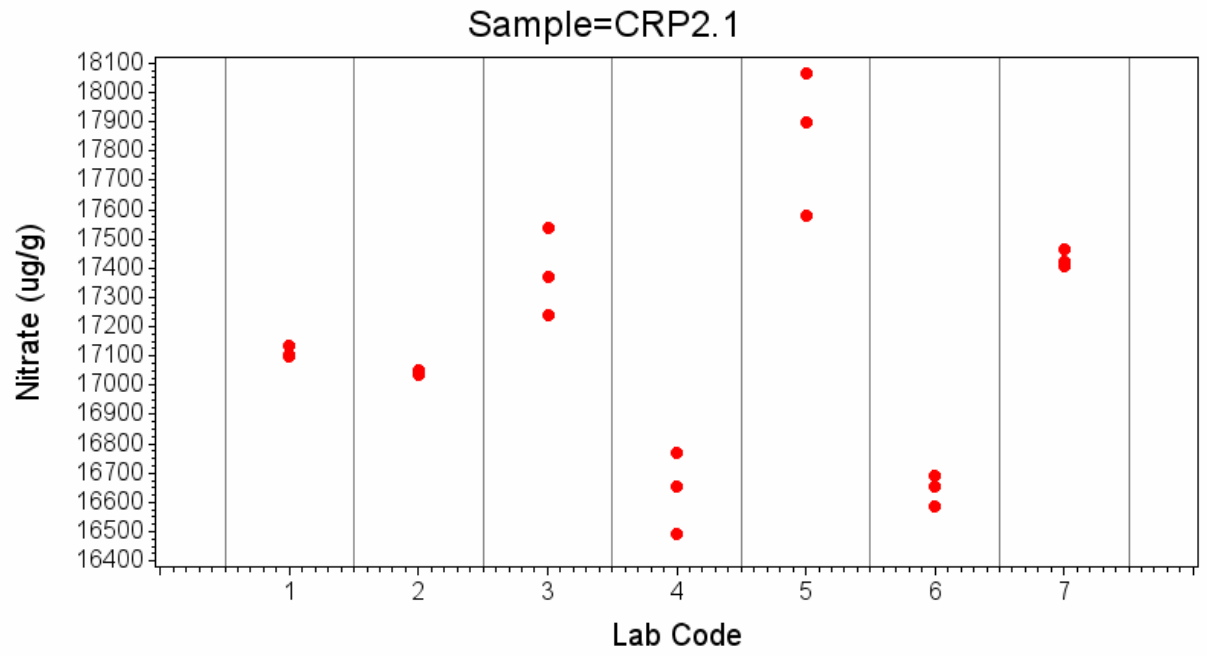
Lab Code	Product	Replicate	Nitrite (µg/g)	LOQ Flag	Nitrate (µg/g)
4	RT1	3	–	<0,6	7077
4	RT3	1	–	<0,6	484
4	RT3	2	–	<0,6	476
4	RT3	3	–	<0,6	490
4	RT6	1	–	<0,6	18622
4	RT6	2	–	<0,6	18542
4	RT6	3	–	<0,6	18581
4	RT8	1	“not analyzed”	NA	“not analyzed”
4	RT8	2	“not analyzed”	NA	“not analyzed”
4	RT8	3	“not analyzed”	NA	“not analyzed”
5	CRP1.1	1	–	<0,47	6014
5	CRP1.1	2	–	<0,46	5940
5	CRP1.1	3	–	<0,44	5911
5	CRP2.1	1	7.81	Above LOQ	18069
5	CRP2.1	2	6.95	Above LOQ	17580
5	CRP2.1	3	7.03	Above LOQ	17897
5	CRP3.1	1	14.65	Above LOQ	36229
5	CRP3.1	2	13.57	Above LOQ	39102
5	CRP3.1	3	15.00	Above LOQ	38509
5	CRP4.1	1	–	<0,67	7507
5	CRP4.1	2	–	<0,68	7647
5	CRP4.1	3	–	<0,67	7721
5	RT1	1	–	<0,68	7273
5	RT1	2	–	<0,68	6945
5	RT1	3	–	<0,68	6984
5	RT3	1	–	<0,67	467
5	RT3	2	–	<0,66	490
5	RT3	3	–	<0,66	488
5	RT6	1	–	<0,67	18496
5	RT6	2	–	<0,67	18152
5	RT6	3	–	<0,67	18187
5	RT8	1	–	<0,68	9093
5	RT8	2	–	<0,68	9076
5	RT8	3	–	<0,67	9006
6	CRP1.1	1	–	<0,60	5723
6	CRP1.1	2	–	<0,60	5719
6	CRP1.1	3	–	<0,60	5723
6	CRP2.1	1	–	<0,60	16589
6	CRP2.1	2	–	<0,60	16691
6	CRP2.1	3	–	<0,60	16655
6	CRP3.1	1	–	<0,60	37973
6	CRP3.1	2	–	<0,60	38639
6	CRP3.1	3	–	<0,60	38431
6	CRP4.1	1	–	<0,60	7169
6	CRP4.1	2	–	<0,60	7186

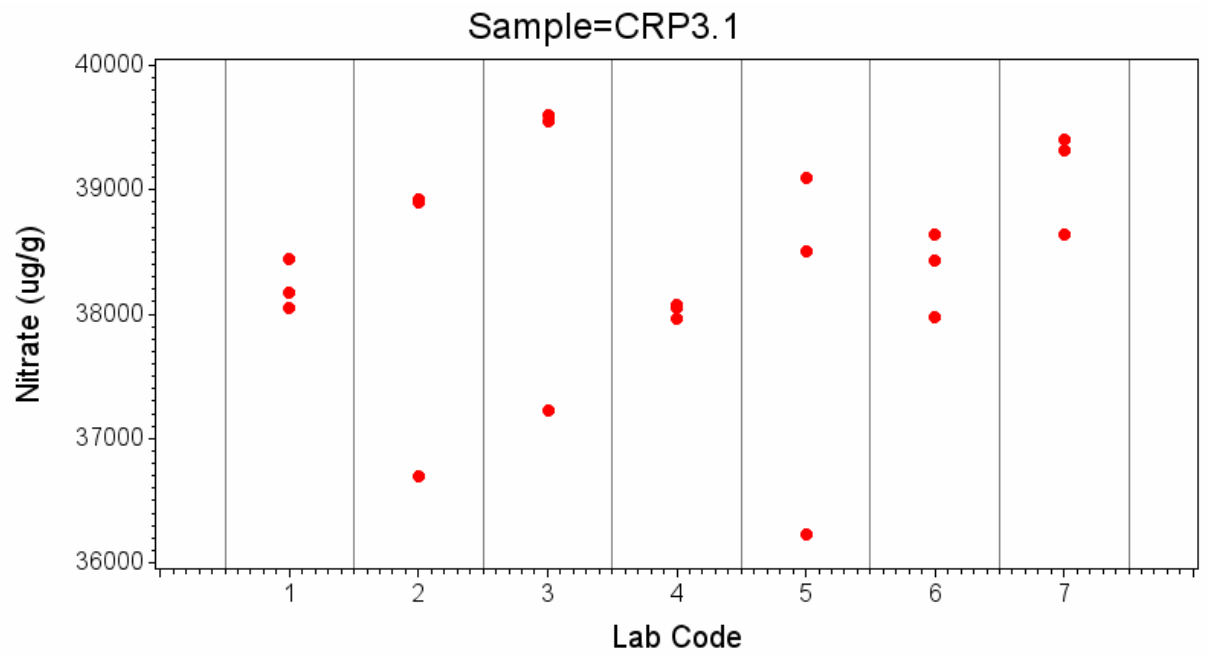
Lab Code	Product	Replicate	Nitrite (µg/g)	LOQ Flag	Nitrate (µg/g)
6	CRP4.1	3	–	<0,60	7161
6	RT1	1	–	<0,60	6713
6	RT1	2	–	<0,60	6814
6	RT1	3	–	<0,60	6814
6	RT3	1	–	<0,60	484
6	RT3	2	–	<0,60	458
6	RT3	3	–	<0,60	454
6	RT6	1	–	<0,60	17636
6	RT6	2	–	<0,60	17535
6	RT6	3	–	<0,60	17599
6	RT8	1	–	<0,60	8731
6	RT8	2	–	<0,60	8640
6	RT8	3	–	<0,60	8756
7	CRP1.1	1	“not analyzed”	NA	5888
7	CRP1.1	2	“not analyzed”	NA	5879
7	CRP1.1	3	“not analyzed”	NA	6027
7	CRP2.1	1	“not analyzed”	NA	17421
7	CRP2.1	2	“not analyzed”	NA	17466
7	CRP2.1	3	“not analyzed”	NA	17406
7	CRP3.1	1	“not analyzed”	NA	38637
7	CRP3.1	2	“not analyzed”	NA	39316
7	CRP3.1	3	“not analyzed”	NA	39403
7	CRP4.1	1	“not analyzed”	NA	7643
7	CRP4.1	2	“not analyzed”	NA	7605
7	CRP4.1	3	“not analyzed”	NA	7628
7	RT1	1	“not analyzed”	NA	7100
7	RT1	2	“not analyzed”	NA	7097
7	RT1	3	“not analyzed”	NA	7099
7	RT3	1	“not analyzed”	NA	525
7	RT3	2	“not analyzed”	NA	508
7	RT3	3	“not analyzed”	NA	510
7	RT6	1	“not analyzed”	NA	18517
7	RT6	2	“not analyzed”	NA	18469
7	RT6	3	“not analyzed”	NA	18504
7	RT8	1	“not analyzed”	NA	9229
7	RT8	2	“not analyzed”	NA	9242
7	RT8	3	“not analyzed”	NA	9245

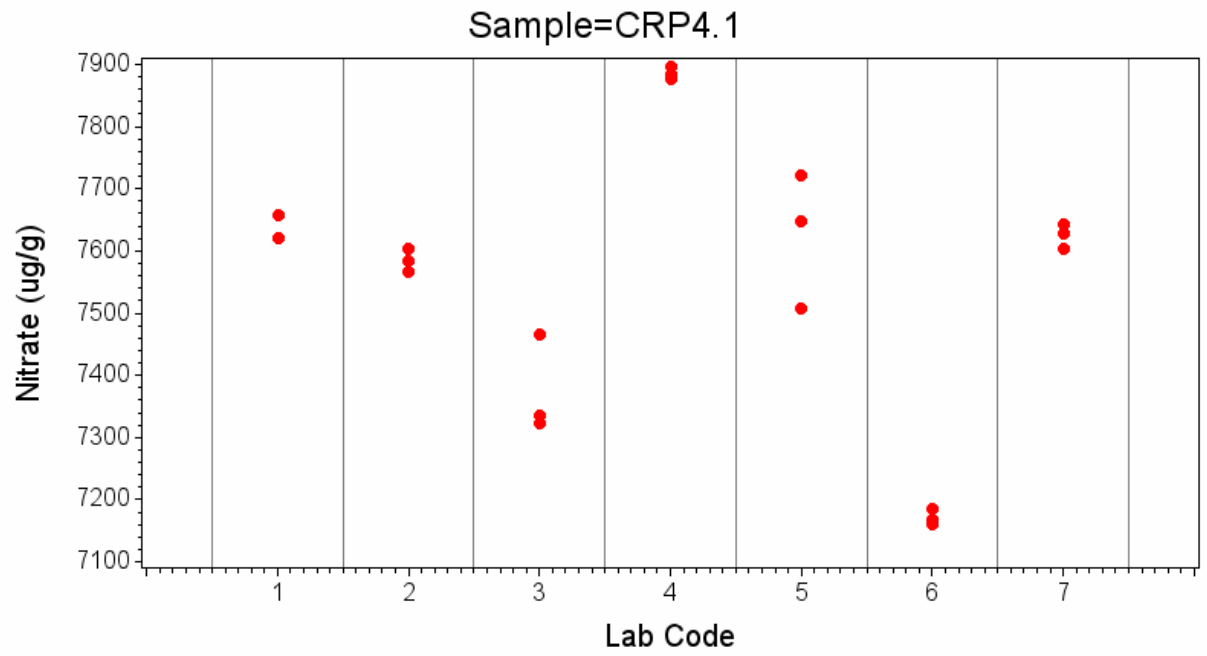
1. The (–) symbol indicates the laboratory did not submit data for that sample analysis.
2. The “<” symbol indicates the result was below the laboratory’s reporting limit

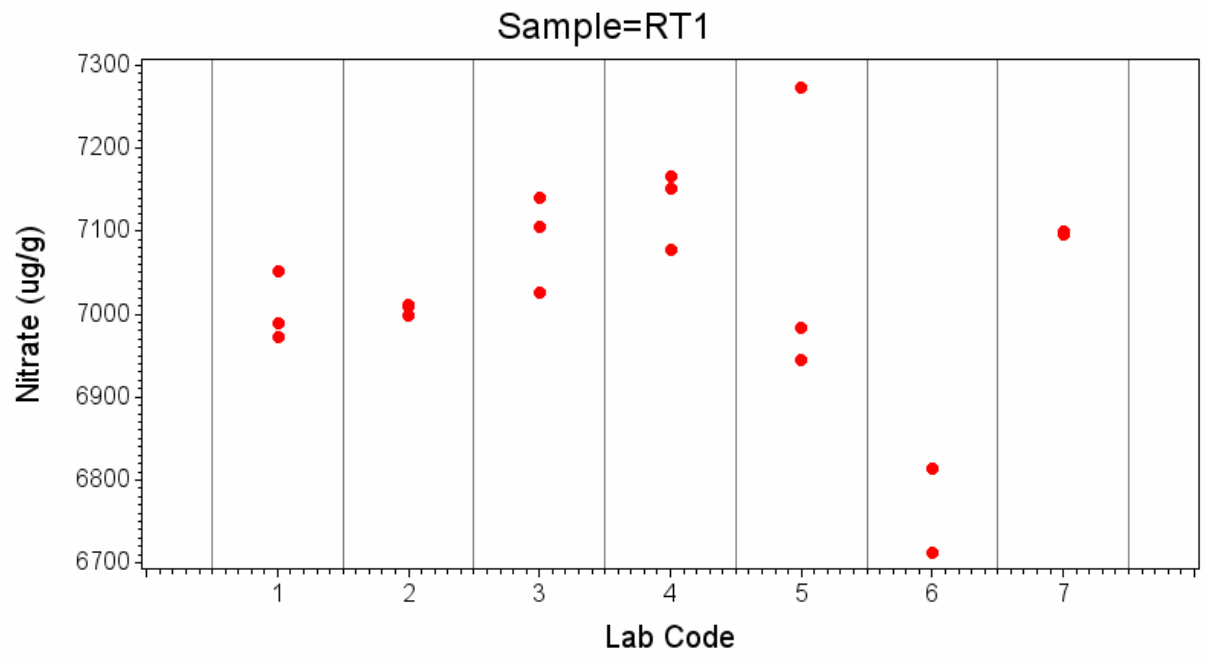
APPENDIX D: Raw Data Plots

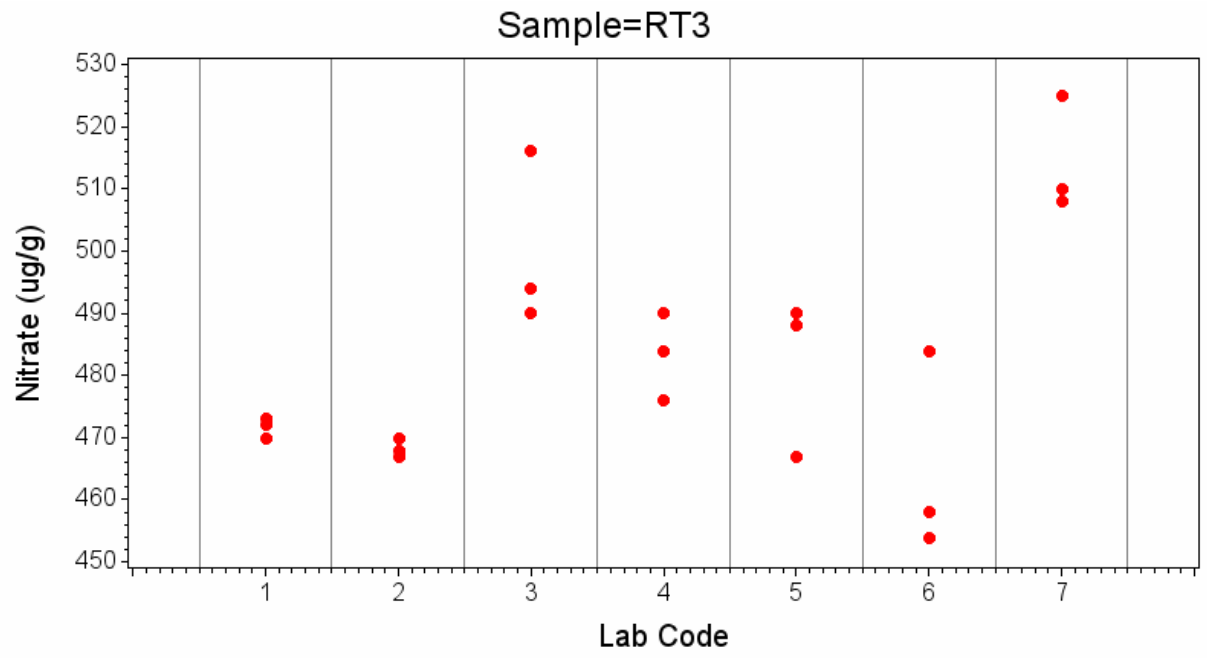


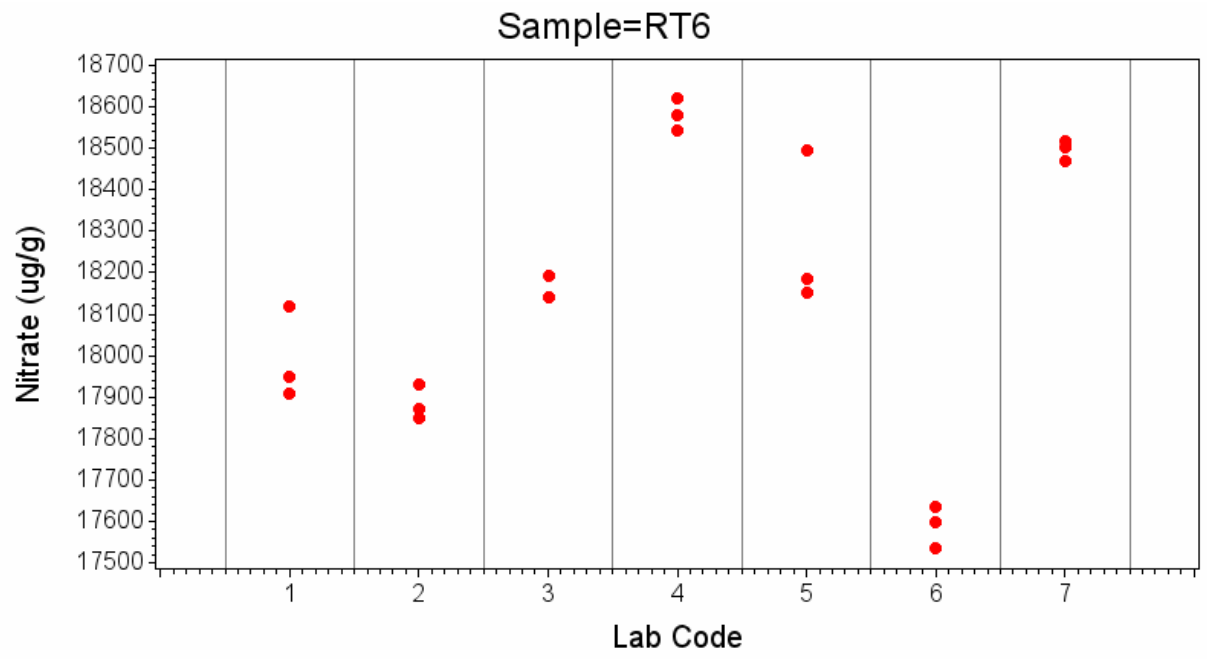


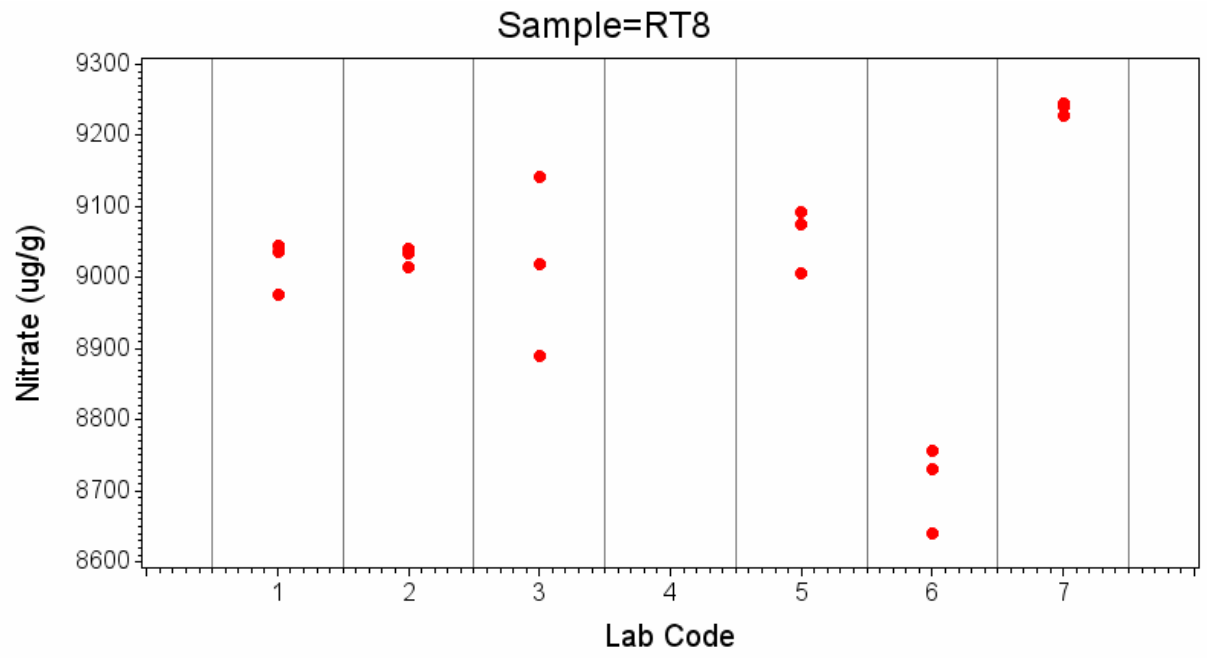

Sample=CRP3.1
Nitrate (ug/g)
40000
39000
38000
37000
36000
1
2
3
4
5
6
7
Lab Code

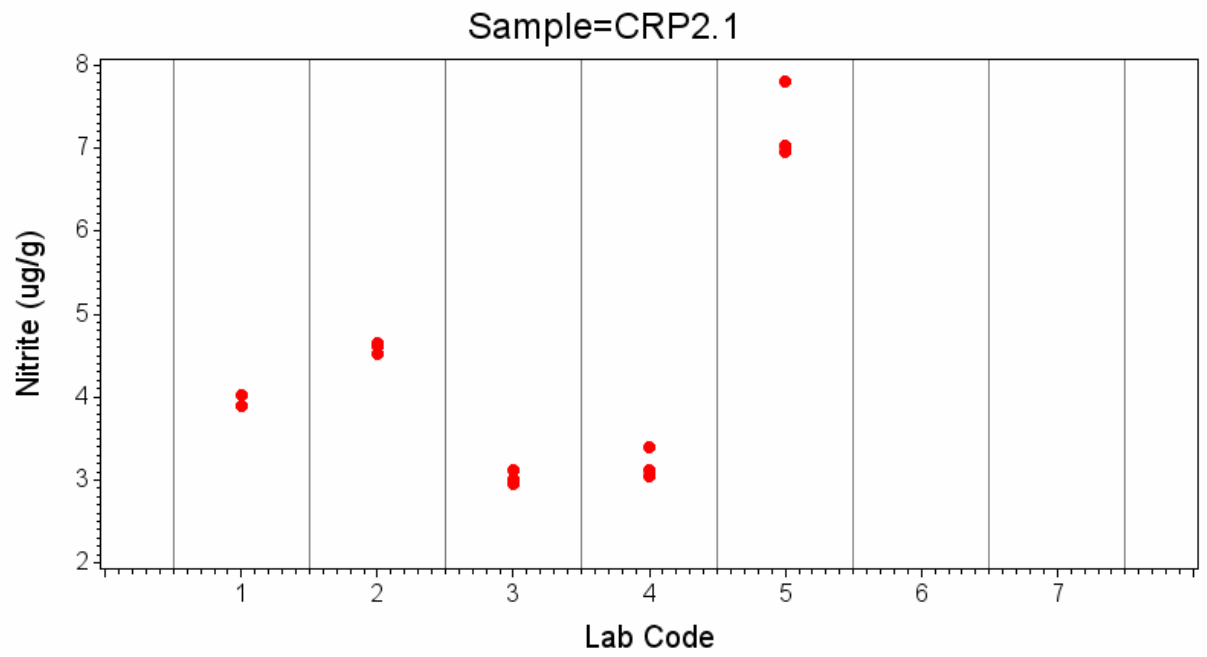

Sample=CRP4.1
Nitrate (ug/g)
7900
7800
7700
7600
7500
7400
7300
7200
7100
1
2
3
4
5
6
7
Lab Code

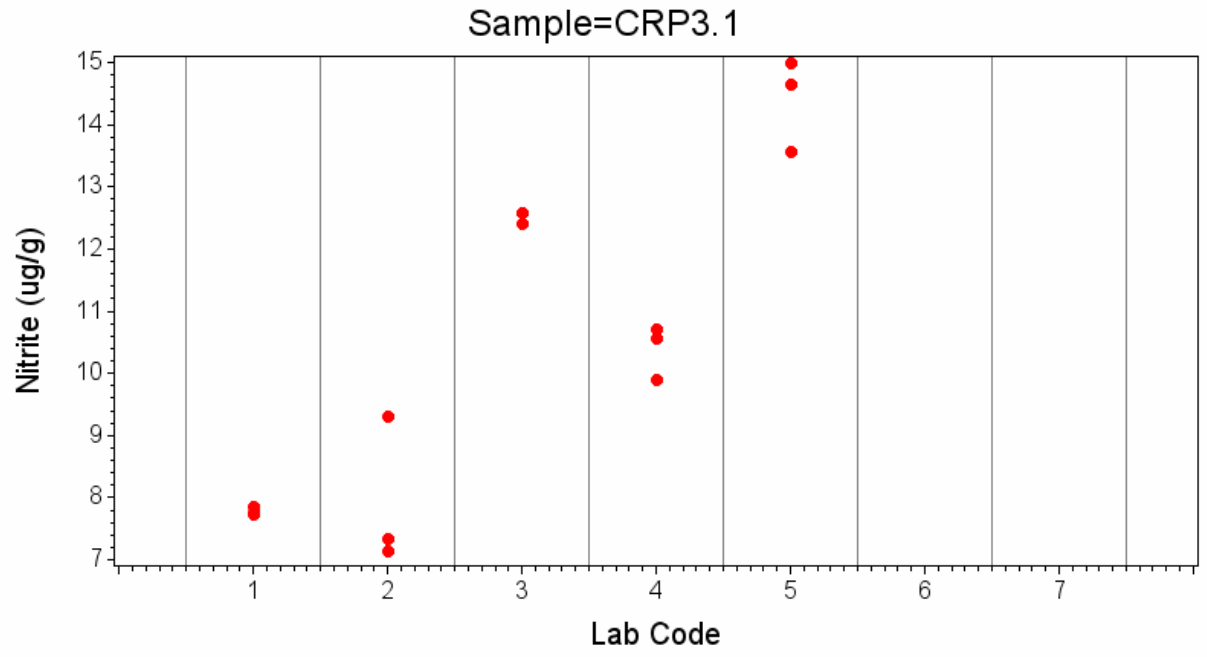

Sample=RT1
Nitrate (ug/g)
7300
7200
7100
7000
6900
6800
6700
1
2
3
4
5
6
7
Lab Code

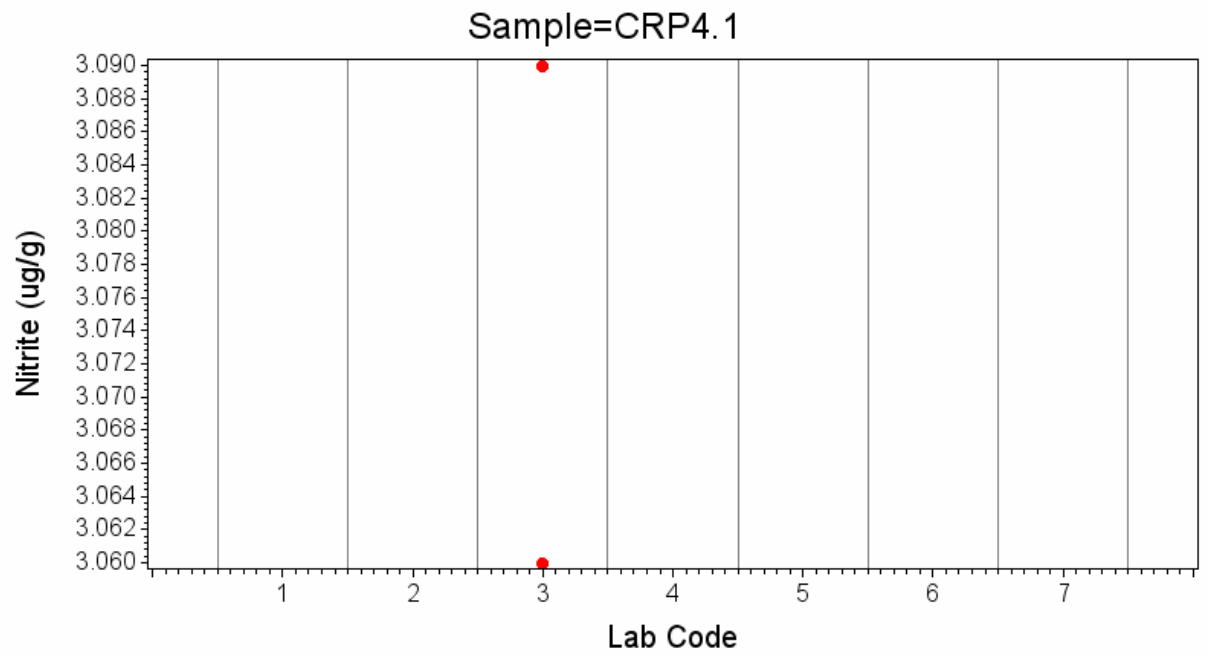

Sample=RT3
Nitrate (ug/g)
530
520
510
500
490
480
470
460
450
1
2
3
4
5
6
7
Lab Code

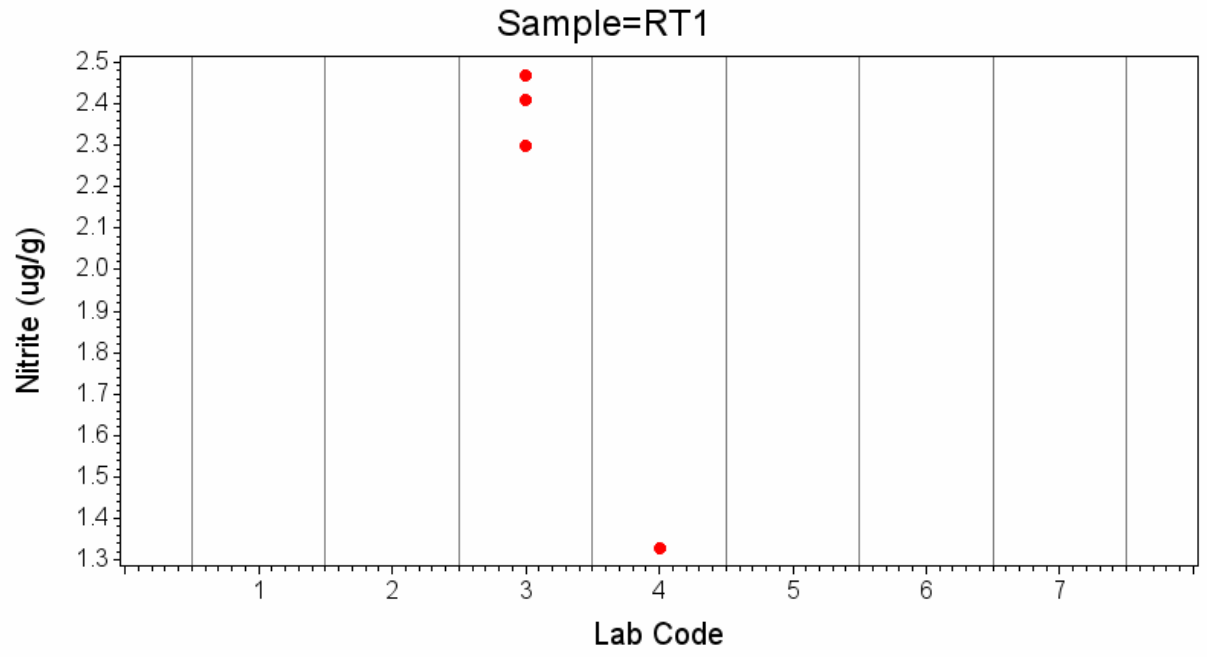

Sample=RT6
Nitrate (ug/g)
18700
18600
18500
18400
18300
18200
18100
18000
17900
17800
17700
17600
17500
1
2
3
4
5
6
7
Lab Code

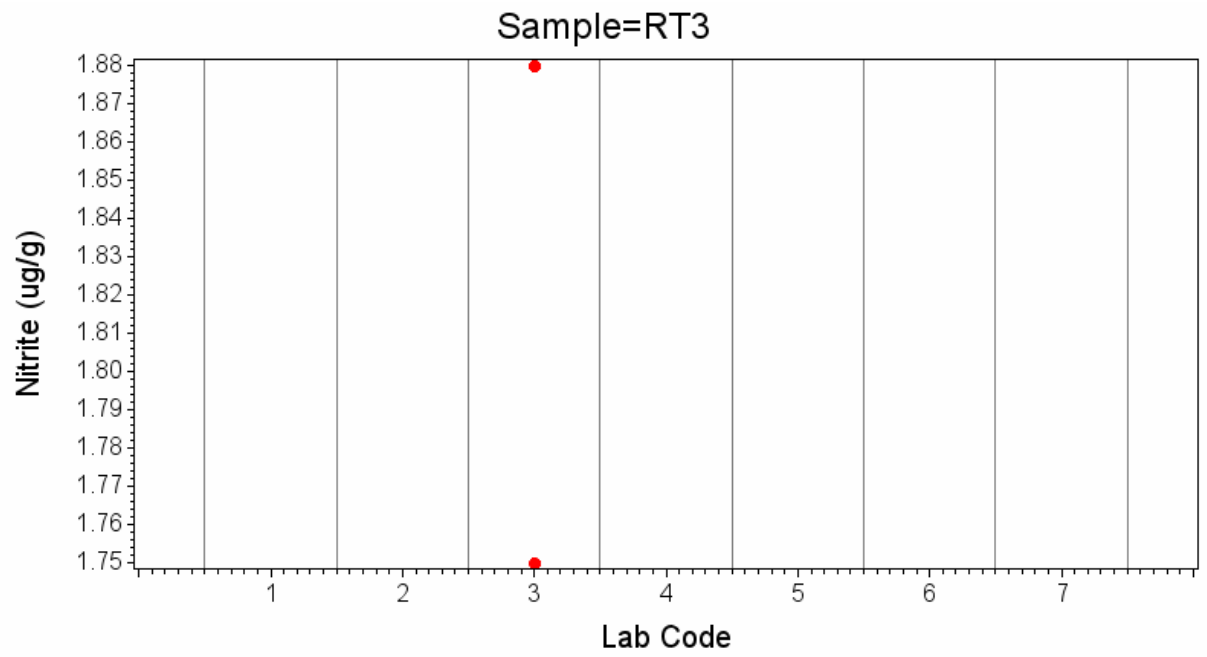

Sample=RT8
Nitrate (ug/g)
9300
9200
9100
9000
8900
8800
8700
8600
1
2
3
4
5
6
7
Lab Code

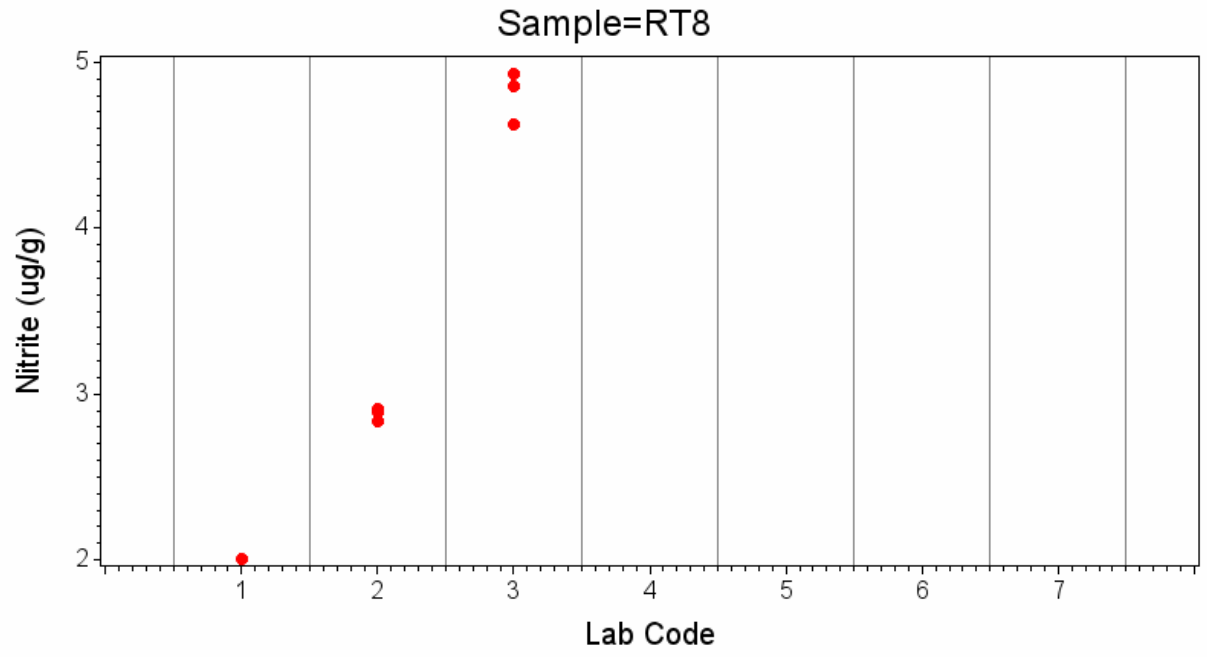

Sample=CRP2.1
Nitrite (ug/g)
8
7
6
5
4
3
2
1
2
3
4
5
6
7
Lab Code

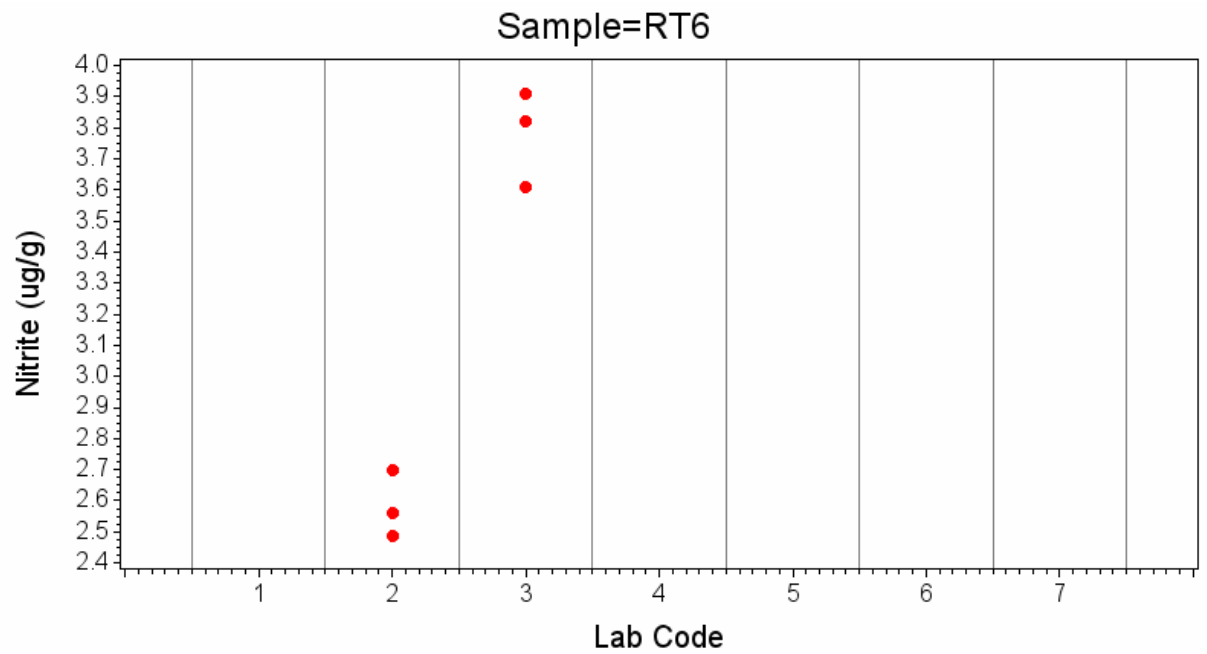

Sample=CRP3.1
Nitrite (ug/g)
15
14
13
12
11
10
9
8
7
1
2
3
4
5
6
7
Lab Code


Sample=CRP4.1
Nitrite (ug/g)
3.090
3.088
3.086
3.084
3.082
3.080
3.078
3.076
3.074
3.072
3.070
3.068
3.066
3.064
3.062
3.060
1
2
3
4
5
6
7
Lab Code


Sample=RT1
Nitrite (ug/g)
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1
2
3
4
5
6
7
Lab Code


Sample=RT3
Nitrite (ug/g)
1.88
1.87
1.86
1.85
1.84
1.83
1.82
1.81
1.80
1.79
1.78
1.77
1.76
1.75
1
2
3
4
5
6
7
Lab Code


Sample=RT8
Nitrite (ug/g)
5
4
3
2
1
2
3
4
5
6
7
Lab Code


Sample=RT6
Nitrite (ug/g)
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
1
2
3
4
5
6
7
Lab Code

APPENDIX E: Z-Scores