Tobacco and Tobacco Products Analysis Sub-Group

Technical Report

Collaborative Study for the Determination of Nicotine Degradants and Impurities in Nicotine Pouches by LC-MS/MS

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1. Summary

At the CORESTA Tobacco and Tobacco Products Analysis Sub-Group (TTPA) meeting held on September 11, 2022, the Sub-Group initiated a collaborative study for the determination of nicotine impurities and degradants in nicotine pouch filler. The intent of this study was to propose a CORESTA Recommended Method (CRM) and to provide repeatability (r) and reproducibility (R) results and z-scores to support laboratory accreditation. Eight laboratories participated in the study.

The results of the study demonstrate that the proposed CRM is suitable for the analysis of nicotine impurities and degradants in nicotine pouch filler samples and the TTPA recommends publication of the CRM.

2. Introduction

Nicotine pouches, or white pouches, are oral tobacco products that contain tobacco derived nicotine, but not tobacco leaf. Currently there are no standardized methods available for the analysis of nicotine degradants and impurities in nicotine pouches. At the CORESTA TTPA meeting held on September 11, 2022, the group agreed to conduct a collaborative study to support the proposal of a CRM for the determination of nicotine impurities and degradants in nicotine pouch filler.

2.1 Objective

The participating laboratories were to provide analytical results for seven nicotine impurities and degradants, including anatabine, anabasine, nicotine-N'-oxide, myosmine, β-nicotyrine, cotinine, and nornicotine.

The study provided an assessment of intra- and inter-laboratory variability for the determination of nicotine impurities and degradants in nicotine pouches using the applicable draft CRM, and thereby confirming the suitability of the draft CRM. Data were collected from the participating laboratories and statistically evaluated in basic conformance with the recommendations of ISO 5725-2:2019 with modifications necessitated by the non-standard design described below. Additionally, z-scores were calculated as a measure of each laboratory's performance as compared to the results of other laboratories

It was not possible to calculate repeatability and reproducibility values for analytes below the limit of detection or quantitation. Some analytes, such as anatabine and anabasine (nicotine impurities), are often not detected in nicotine pouches or are below the limit of quantitation. To demonstrate that the draft CRM is fit for use for the determination of all seven analytes in nicotine pouch filler, five out of the eight participating laboratories conducted additional fortification studies.

3. Organization

3.1 Participants

A list of the participating laboratories is provided in Table 1. All laboratories provided data for all seven analytes. However, three laboratories did not provide Fortified Filler-1-1 sample data. The laboratories are listed in alphabetical order. Numerical 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
Enthalpy Analytical, Richmond, United States
Imperial Brands Reemtsma, Germany
JTI Oekolab, Austria
Labstat International ULC, Canada
R.J. Reynolds Tobacco Company, United States
Swedish Match Northern Europe, Sweden
University of Kentucky, United States

3.2 Protocol

The protocol is provided in Appendix A and specific details from the protocol are described below. The draft CRM specified in the protocol is included in Appendix B.

3.2.1 Sample Shipment

The samples listed in Table 2 were distributed by Swedish Match, Altria, and American snuff. Filler samples were shipped at ambient conditions in polypropylene tubes. Upon receipt, the laboratories were requested to store the samples at approximately −20 °C. Laboratories were requested to conduct the study in February and report data by March 15, 2023.

Table 2: Sample identification

Sample	Sample Description	Distributed by	Nicotine (mg/g)	OV (%)
Filler-1	Artificially aged dry nicotine pouch filler	Swedish Match	6	3
Filler-2	Artificially aged wet nicotine pouch filler	Swedish Match	1,4	41
Filler-3	Artificially aged dry nicotine pouch filler	Altria	32	6
Filler-4	Artificially aged wet nicotine pouch filler	American Snuff	11	15
Fortified Filler-1-1[1]	Artificially aged dry nicotine pouch filler	Swedish Match	6	3

Filler-1 was fortified and extracted to be used as a quality check during the study (Fortified Filler-1-1).

3.2.2 Sample Analysis and Data Reporting

The participating laboratories were instructed to conduct triplicate analyses on 4 days using two analysts where each replicate represented an individual sample weighing and preparation (total n = 3 replicates per day * 4 days = 12). Participating laboratories were requested to document any deviations from the protocol and the draft CRM and submit the deviations with their results. As stated in the protocol, data submitted with significant deviations from the draft CRM were excluded from the r&R portion of the study. Deviations reported by the laboratories are listed below as well as if the data were excluded from the study.

- Lab 3: Minor deviations. Nicotine-N'-oxide was quantitated with Cotinine-d3 as the internal standard. Instrument conditions were modified to achieve resolution and sensitivity. This included gradient, column temperature, transitions, flow and injection volume.
- Lab 3: Major error. Nornicotine values in day 2, 3, and 4 were very low due to fortification standards preparation issue with this analyte.
- Lab 5: Minor deviations. Anatabine-d4 internal standard was used for the quantitation of β-nicotyrine due to poor chromatographic peaks obtained for β-nicotyrine-d3 internal standard. Sample preparation error occurred with Filler-1 on day 4 for replicate 3. Internal standard area was found to be ~1.5× more than other replicates for all analytes, suggesting an addition error of internal standard amount in this sample which caused falsely low results. The data from this laboratory were dropped from the r&R calculations.
- Lab 7: Minor deviations. 20 µL of 10 µg/mL mixed internal standard solution (ISTD) was added after sample extraction to 980 µL of the extract instead of using 60 µL of 100 µg/mL mixed internal standard solution (ISTD) prior to the extraction.
- Lab 8: Major deviations. MeOH was used as a Mobile B instead of Acetonitrile and 2 µL was used instead if 1 µL. Samples weight ranged from 0,25-0,35 g in 15 mL extraction volume instead of 1 g in 30 mL extraction volume. The data from this laboratory were dropped from the r&R calculations.

All test results were to be reported in µg/g to three decimal places. The study results and the comments were to be sent by e-mail to the study coordinator (Fadi Aldeek).

4. Data – Raw

The full data set for the study is provided in Appendix C. The results are presented on an as-is basis. Each analysis includes three replicates on each analysis day. As mentioned above, data sets were removed from the r&R portion of the study when identified as outlying data. Those data are included in Appendix C but were eliminated prior to the r&R analysis. Raw data plots are provided in Appendix D. As with Appendix C, the raw data plots include outliers.

5. Statistical Analysis

The statistical analysis was conducted in basic conformance with ISO 5725-2:2019 with necessary modifications based on the design of the study. The study was conducted over four days at each laboratory as described above. The intent of that was to treat each day of testing for each lab as if it were a separate laboratory to ensure sufficient data were available for the calculation of r & R. However, in most instances, the data did not support treating the data in that manner because the variability between days within the laboratories was typically significantly (both statistically and from a practical perspective) less than the variability between laboratories. The standard model for a collaborative study is a one-way random effects analysis of variance model associated with each analyte:

$$y_{ij} = m + L_i + e_{ij}$$

where m is the mean value, L_i is a random laboratory effect associated with laboratory i and e_{ij} is the random effect associated with j-th replicate in the i-th laboratory. In this case, the testing in a single lab is typically within a single day and the day-to-day effect is not formally included in the model but is implicitly contained in the laboratory effect. In this study, with multiple days of testing, day-to-day variation must be explicitly separated out and the model is:

$$y_{ijk} = m + L_i + d_{ij} + e_{ijk}$$

In this situation model, the d_{ij} are the day-to-day effects within each laboratory and now the interpretation of the L_i is that a representation of the lab-to-lab variation over and above the day-to-day effects. In this case, treating the day-to-day effects as if they were separate laboratories would only be appropriate if the estimated lab-to-lab effects were small in comparison to the day-to-day effects (literally only if the L_i effects were zero, but the approximation would be reasonable if the additional lab-to-lab effects were "small" in comparison with the day-to-day effects).

A summary of the analysis of variance table comparing day-to-day variation and between laboratory variation after the removal of outliers is shown in Table 3. In each case there was additional variation between laboratories over and above the day-to-day within laboratories variation and in most cases that difference was of a practical importance. In the r&R tables below, what is normally simply lab-to-lab variation is replaced by a sum of the lab-to-lab and day-to-day variation. Since the variation is composed of two separate components, the reliability of the estimate is improved relative to what it would have been if only one day of testing per lab had been completed. That is estimated in the r&R table in section 5.2 below and is called the "effective number of laboratories" and is carried out using the Satterthwaite approximation. This is an approximation employed when two or more sources of variation are combined.

Table 3. Analysis of variance table examining lab-to-lab vs. day-to-day variation

Sample	Analyte	Lab MS	Day MS	F-Ratio	P-value
Fortified Filler-1	Anabasine	1,34	0,44	3,045	0,0532
Fortified Filler-1	Anatabine	4,24	0,55	7,709	0,0017
Filler-2	β-Nicotyrine	0,14	0,02	7,000	0,0011
Filler-3	β-Nicotyrine	0,17	0,01	17,000	<0,0001
Fortified Filler-1	β-Nicotyrine	13,97	0,57	24,509	<0,0001
Filler-1	Cotinine	0,37	0,01	37,000	<0,0001
Filler-2	Cotinine	2,32	0,31	7,484	0,0003
Filler-3	Cotinine	1,06	0,06	17,667	<0,0001
Filler-4	Cotinine	6.72	0.09	74.667	<0,0001
Fortified Filler-1	Cotinine	2,1	0,39	5,385	0,0077
Filler-2	Myosmine	0,36	0,08	4,500	0,0048
Filler-3	Myosmine	0,27	0,02	13,500	<0,0001

Sample	Analyte	Lab MS	Day MS	F-Ratio	P-value
Filler-4	Myosmine	0,36	0,01	36,000	<0,0001
Fortified Filler-1	Myosmine	2.08	0.38	5.474	0.0072
Filler-1	Nicotine-N'-Oxide	31,69	1,81	17,508	<0,0001
Filler-2	Nicotine-N'-Oxide	65877	3044	21,643	<0,0001
Filler-3	Nicotine-N'-Oxide	1635	68,8	23,767	<0,0001
Filler-4	Nicotine-N'-Oxide	31776	1311	24,238	<0,0001
Fortified Filler-1	Nicotine-N'-Oxide	34,97	1,75	19,983	<0,0001
Filler-1	Nornicotine	4,79	0,35	13,686	<0,0001
Filler-2	Nornicotine	38	3,75	10,133	<0,0001
Filler-3	Nornicotine	28,84	3,82	7,550	0,0002
Filler-4	Nornicotine	24,21	2,63	9,205	<0,0001
Fortified Filler-1	Nornicotine	12,92	0,39	33,454	<0,0001

Lab MS and Day MS are the mean squares for those effects, F-ratio is the ratio of the two, and the p-value is judged statistically significant if $p<0,05$.

Outliers were calculated using Grubbs' Test and Cochran's Test applied to each day of testing within each lab. This was done for the purpose of outlier detection, but treated differently for the r&R calculations, since there is no standardized method for outlier detection exactly fitting this situation. The iterative application of the outlier detection tests was stopped after two outliers were identified for the same analyte on the same product.

5.1 Exclusion of Outliers

As noted above Grubbs' Test and Cochran's Test were employed to identify outliers. The tests were applied to the data set consisting of the means and standard deviations for each day within each laboratory. The tests were applied iteratively, but only to the point that up to two outliers were identified for the same analyte for the same product. There were no Grubbs' outliers and the Cochran's test outliers are shown in Table 4.

Table 4: Outliers

Analyte	Product	Grubbs' Outlier Lab	Cochran's Outlie Lab
Anabasine	Fortified Filler-1	--	Lab 3 Days 1* and 4
Anatabine	Fortified Filler-1	--	Lab 3 Day 4
β-Nicotyrine	Filler 3	--	Lab 7 Days 1 and 3
β-Nicotyrine	Fortified Filler-1	--	Lab 3 Day 4
Cotinine	Filler 2	--	Lab 7 Day 2
Cotinine	Fortified Filler-1	--	Lab 3 Day 4
Myosmine	Filler 2	--	Lab 7 Day 2
Myosmine	Filler 3	--	Lab 5 Days 1 and 2
Myosmine	Filler 4	--	Lab 7 Day 4
Myosmine	Fortified Filler-1	--	Lab 3 Day 4
Nicotine-N'-Oxide	Filler 1	--	Lab 7 Days 2 and 4
Nicotine-N'-Oxide	Filler 4	--	Lab 7 Days 2 and 4
Nicotine-N'-Oxide	Fortified Filler-1	--	Lab 3 Days 1 and 4
Nornicotine	Filler 1	--	Lab 3 Day 4
Nornicotine	Filler 2	--	Lab 7 Day 2
Nornicotine	Filler 4	--	Lab 4 Day 1

"*" indicates Day 1 was treated as a single-point (i.e., replicate) outlier and the value of 17,203 was removed.
"--" indicates no outliers were identified

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

After removal of outlying data based on numerical data consistency methods (Grubbs' Test and Cochran's Test), the final repeatability and reproducibility (r & R) results were calculated. The r&R results are shown in the table below. The r&R results reflect both laboratory variability and product consistency. For comparison we also include modeled R % as calculated from the Horwitz-Thompson (HT) equation as a benchmark comparison. The results from our study are comparable to or a little better than the HT equation predictions.

Table 5: Repeatability (r) and reproducibility (R) results

Analyte	Product	Mean (µg/g)	N Labs*	Eff. N Labs#	r (µg/g)	r %	R (µg/g)	R %	HT Equation R %
Fortified Filler-1	Anabasine	9,98	5	16	0,950	9,5 %	1,55	15,5 %	31,7 %
Fortified Filler-1	Anatabine	10,32	5	8	1,110	10,7 %	2,19	21,2 %	31,5 %
Filler-2	β-Nicotyrine	0,97	7	11	0,205	21,0 %	0,43	43,7 %	45,0 %
Filler-3	β-Nicotyrine	1,13	6	7	0,203	18,0 %	0,41	36,5 %	44,0 %
Fortified Filler-1	β-Nicotyrine	9,74	5	6	0,957	9,8 %	3,37	34,6 %	31,8 %
Filler-1	Cotinine	1,10	7	9	0,142	12,8 %	0,55	49,5 %	44,1 %
Filler-2	Cotinine	3,42	7	15	0,241	7,1 %	1,49	43,4 %	37,2 %
Filler-3	Cotinine	2,12	7	10	0,245	11,6 %	0,92	43,5 %	40,0 %
Filler-4	Cotinine	6,01	7	7	0,494	8,2 %	2,17	36,2 %	34,2 %
Fortified Filler-1	Cotinine	11,10	5	11	0,932	8,4 %	1,67	15,0 %	31,2 %
Filler-2	Myosmine	1,96	7	19	0,200	10,2 %	0,65	33,2 %	40,5 %
Filler-3	Myosmine	1,12	7	10	0,208	18,5 %	0,51	45,2 %	44,0 %
Filler-4	Myosmine	1,34	7	8	0,176	13,1 %	0,54	40,0 %	42,9 %
Fortified Filler-1	Myosmine	10,56	5	11	0,866	8,2 %	1,63	15,5 %	31,4 %
Filler-1	Nicotine-N'-Oxide	10,50	7	10	1,19	11,3 %	5,21	49,6 %	31,4 %
Filler-2	Nicotine-N'-Oxide	435,6	7	9	30,5	7,0 %	222,8	51,1 %	17,9 %
Filler-3	Nicotine-N'-Oxide	52,71	7	9	6,35	12,0 %	35,07	66,5 %	24,7 %
Filler-4	Nicotine-N'-Oxide	354,0	7	9	25,8	7,3 %	159,5	45,0 %	18,5 %
Fortified Filler-1	Nicotine-N'-Oxide	20,37	5	5	3,41	16,8 %	6,06	29,8 %	28,5 %
Filler-1	Nornicotine	2,62	7	11	0,384	14,6 %	2,02	76,9 %	38,7 %
Filler-2	Nornicotine	12,03	7	12	1,083	9,0 %	5,82	48,3 %	30,8 %
Filler-3	Nornicotine	11,33	7	14	1,484	13,1 %	5,27	46,5 %	31,1 %
Filler-4	Nornicotine	6,05	7	13	0,637	10,5 %	4,67	77,2 %	34,2 %
Fortified Filler-1	Nornicotine	12,62	5	13	1,023	8,1 %	3,41	27,0 %	30,6 %

*** This is the number of laboratory data sets with reported values after removal of outliers.**

The "effective" number of labs is an approximation to the equivalent number of labs involved taking into account the additional information coming from having multiple days of testing within each lab. This was calculated using the Satterthwaite approximation. The calculated values are fractions but were rounded to integers.

The Horwitz equation in this range of concentrations is $5.6 \cdot C^{-0,1505}$, where C is the analyte concentration. This form of the equation is intended to predict the reproducibility limit of the analytical method and can be used as a rough benchmark for analytical variation.

5.3 Calculation of Z-Scores

The calculation of z-scores is not required to fulfill the objectives of the study but was conducted as a courtesy to the participating laboratories to aid in their accreditation. As mentioned above, data sets were removed from the r&R portion of the study due to significant deviations from the study protocol or if the data were identified as outlying data. Those data are included in the calculation of z-scores.

The z-scores were calculated using methods suggested in ISO 13528: 2005. The calculations were based on Algorithm A given in Annex C of ISO 13528: 2005. It is expected that most of the data should fall within the range of ±2, and that laboratories having values with $|z| \geq 3$ should be treated as an "action signal" to investigate laboratory performance. Values in the interval $2 \leq |z| < 3$ should be treated as a warning signal. Final summary tables of z-scores are presented in the table below. Graphs of the z-scores are presented in Appendix E.

Table 6: Z-scores

Laboratory	Filler-1	Filler-2	Filler-3	Filler-4	Fortified Filler-1
Anabasine					
1	–	–	–	–	0,12
2	–	–	–	–	-2,11
3	–	–	–	–	0,34
4	–	–	–	–	0,21
5	–	–	–	–	0,83
6	–	–	–	–	–
7	–	–	–	–	–
8	–	–	–	–	–
Anatabine					
1	–	–	–	–	0,71
2	–	–	–	–	-1,20
3	–	–	–	–	0,82
4	–	–	–	–	0,30
5	–	–	–	–	-0,63
6	–	–	–	–	–
7	–	–	–	–	–
8	–	–	–	–	–
β-Nicotyrine					
1	–	-1,19	-0,99	–	0,71
2	–	0,21	0,10	–	0,36
3	–	-0,41	-0,19	–	0,47
4	–	-0,97	-1,19	–	-0,05
5	–	0,84	–	–	-2,26
6	–	0,47	0,22	–	–
7	–	-0,31	1,21	–	–
8	–	1,36	0,84	–	–

Laboratory	Filler-1	Filler-2	Filler-3	Filler-4	Fortified Filler-1
Cotinine					
1	-0,11	1,27	0,47	1,26	1,11
2	1,22	0,55	0,66	0,51	-0,57
3	0,32	0,03	-0,49	-0,29	0,70
4	-0,19	0,27	0,46	0,54	-0,25
5	-0,29	-0,74	-0,26	0,16	-1,00
6	-0,72	-0,51	-0,62	-0,94	–
7	-1,38	-1,92	-1,53	-2,47	–
8	1,15	0,63	1,28	0,26	–
Myosmine					
1	–	0,50	-0,45	0,49	0,90
2	–	-0,15	0,48	-0,57	-1,15
3	–	0,61	0,84	1,20	0,64
4	–	-0,56	-0,97	-0,58	-0,70
5	–	-0,74	0,55	0,23	0,30
6	–	-1,55	-1,40	-2,31	–
7	–	1,00	-0,10	0,89	–
8	–	0,83	1,05	-0,15	–
Nicotine-N'-Oxide					
1	0,26	0,37	0,20	0,28	0,44
2	-0,04	-0,32	-0,17	-0,13	-0,21
3	-1,44	-1,03	-1,98	-1,12	1,08
4	-0,02	-0,21	-0,07	-0,19	-0,01
5	-1,14	-1,34	-0,92	-1,25	-1,31
6	0,57	0,90	0,62	0,83	–
7	1,09	1,09	1,23	1,31	–
8	0,72	0,54	0,62	0,27	–
Nornicotine					
1	-0,28	1,01	-0,21	-0,06	-0,14
2	-0,20	-0,81	-0,66	-0,56	-0,73
3	1,28	1,14	1,39	1,26	1,46
4	1,95	0,57	0,82	1,90	0,05
5	-0,80	-1,26	-1,35	-0,60	-0,64
6	-0,55	-0,72	-0,34	-0,63	–
7	-0,35	-0,02	-0,25	-0,19	–
8	-0,62	0,09	0,60	-0,71	–

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

6. Data Interpretations

A commonly used benchmark for collaborative studies is the HT equation.[1] The reproducibility values in this study were generally in line with the HT equation and from that perspective are in a reasonable range for a CORESTA Recommended Method.

7. Recommendations

The results of this study demonstrate that the draft CRM is suitable for the analysis of nicotine impurities and degradants in nicotine pouch filler and the TTPA recommends publication of the draft CRM as a new CORESTA Recommended Method.

[1] See "Horwitz-Thompson Equation as a Benchmark for CORESTA Collaborative Study Results" Michael Morton, presentation #52, TSRC in Norfolk, Virginia, U.S.A..

APPENDIX A: Protocol

CORESTA TOBACCO and TOBACCO PRODUCTS ANALYSIS (TTPA) SUB-GROUP

Collaborative Study for the Determination of Nicotine Degradants in Nicotine Pouches

Type of Document: Collaborative Study Protocol

November 2022, Version 2

Study Coordinator: Fadi Aldeek, Ph.D.

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

1. Objective

The objective of this collaborative study is to develop a CORESTA Recommended Method (CRM) for the determination of seven nicotine degradants and impurities in nicotine pouch products. Nicotine pouches are pre-portioned products like snus but replace the tobacco leaf with non-tobacco filler and tobacco-derived nicotine.

2. Analytes and Method

The draft CRM shall be used for the determination of the nicotine degradants. Data collected with other methods, or with significant deviations from the draft CRM cannot be included in the study since the purpose of the method is to calculate method repeatability and reproducibility values. The seven nicotine degradants include anatabine, anabasine, nicotine-N'-oxide, myosmine, β-nicotyrine, cotinine, and nornicotine. The CAS Registry numbers for the analytes are provided in the draft CRM. Calibration standards can be purchased as individual neat materials or a primary stock mixed solution. For example: ISO 17034 standards mix of the seven nicotine degradants in Methanol at 1000 µg/mL can be purchased form SPEX Certiprep or LGC Standards (Cat# G34-113070-01-10PAK). The corresponding isotopically labelled internal neat standards can be individually purchased from TRC.

3. Samples

The description of the samples is given in the table below.

Sample	Product Characterisation	Distributed by
Filler-1	Artificially aged dry nicotine pouch filler	Swedish Match
Filler-2	Artificially aged wet nicotine pouch filler	Swedish Match
Filler-3	Artificially aged dry nicotine pouch filler	Altria
Filler-4	Artificially aged wet nicotine pouch filler	American Snuff

The samples will be distributed in January 2023. In case laboratories do not receive the samples, please contact the study coordinators (Fadi Aldeek).

4. Samples Shipments and Receipt

Each participating laboratory will need to send their shipping address, person to whom delivery should be made, shipping account (FedEx International, DHS, UPS) arrangements and any special delivery information to the study coordinator, Fadi Aldeek. The study coordinator will inform the laboratories of the actual shipping date and tracking information so that the receiving laboratories can prepare for receipt of the samples. Samples will be shipped at ambient conditions. Laboratories should report any observed issue on the received samples and should contact the study coordinator if they question the integrity of the samples they received.

Each participating laboratory will receive approximately 50 g of filler for each of the above samples. Samples will be received in polypropylene tubes. Upon receipt, the samples shall be immediately stored at the recommended long-term storage temperature of -20 °C. Samples analyzed within one week may be stored in the refrigerator at approximately 4 °C.

5. Feasibility Experiment

Once the samples are received by the participating laboratories, we first recommend the laboratories to check the feasibility of the draft CRM method to make sure each laboratory can perform the method with no issue. For this, Filler-1 sample will be analyzed in triplicate. The results (µg/g) and chromatograms will be sent to the study coordinator, Fadi Aldeek, for a quick evaluation before the laboratories officially start the 4 days study. Laboratories should report any observed issue on the obtained data and should contact the study coordinator if they question the chromatography or the quality of the data they obtained.

6. Samples Analysis

6.1 **Sample Preparation:** 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 and placed at laboratory conditions 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 shall be stored at approximately 4 °C for up to one month if the analyses will not be conducted immediately.

6.2 **Sample Analysis:** Each sample shall be analysed in triplicate on each day (n=3), over four different days (n=12), and by two different analysts, two days per analyst. The four days analyzes shall be conducted within a month. Store the samples in the refrigerator when not in use.

6.3 **Quality Control:** Since a certified reference material (CRM) is not available, a fortified and extracted sample will be used as a quality check in each batch to determine if the analytical method accurately measures the concentration of the nicotine degradants in the presence of sample matrix components. Filler-1 sample will be fortified with 100 µL (100 µg/mL) solution in triplicate (n=3), extracted following the draft CRM, and analyzed on each day of analysis (4 days, n=12). The resulting analyte concentrations in the fortified sample must remain within the calibration range stated in the draft CRM. Three replicates of filler-1 unfortified sample will also be prepared. Any amount of nicotine degradants identified in the average of the three unfortified samples replicates will be subtracted from the results of the fortified samples results before percent recovery (% recovery) is calculated. The analyte concentrations determined in the unfortified samples and the fortified samples will be presented as well as the target fortification concentrations. Individual and mean % recovery, standard deviation, and %RSD will be calculated and presented. Accuracy will be calculated with the following equation:

$$\% \text{ Recovery} = (\text{Fortified Sample Conc.} - \text{Unfortified Sample Conc.}/\text{Concentration of Fortification}) \times 100\ \%$$

Where:

Fortified Sample Conc. = The analyte concentration determined experimentally in the fortified matrix.

Unfortified Sample Conc. = The experimentally determined analyte concentration of the sample before fortification.

Concentration of Fortification = The theoretical concentration of the fortification in the resulting sample.

Acceptance Criteria: % recovery values of the average of three replicates must be between 75 % and 125 %.

7. Data Reporting

The provided data reporting spreadsheet with the results of the four days analyzes shall be sent by email to the study coordinator, Fadi Aldeek.

Data shall be reported in the units and number of significant figures specified in the Data Reporting Sheet (in µg/g to three decimal places).

If data are below a Limit of Quantitation (LOQ), the numerical LOQ shall be stated preceded by a "<" symbol.

Please provide any deviation of the method or any unusual observation during analysis of the samples.

8. Statistical Analysis

Within and between laboratory variability will be evaluated in basic conformance with the recommendations of ISO 5725-2:2019 or ISO 5725-5:1998. Additionally, z-scores will be calculated to allow laboratories to compare their results to those of other laboratories.

9. Study Timeline

- November 2022

  The following documents will be distributed to participating laboratories
  - Protocol of the study (This document)
  - Report form
  - Draft CRM
- January 2023

  Products shipment. Samples will be shipped to the laboratories.
- February 2023

  Laboratories will perform the study.
- March 15th, 2023

  The results must be sent to the data coordinator (Fadi Aldeek) to give to the statistician (Michael Morton) enough time to evaluate the data.
- March 30th, 2023

  The statisticians will perform the data analysis and SG coordinator format the data for presentation.
- April or May 2023

  The SMA SG will assess the results of the CS during the TTPA SG meeting in somewhere.

10. Tabulation and Presentation of the Data

The data will be coded, and the code will be provided to the respective participating laboratory along with the tabulated data. Results will be presented at the spring 2023 TTPA meeting, and a technical report will be drafted.

APPENDIX B: Draft CRM

DRAFT CORESTA RECOMMENDED METHOD N° 105

DETERMINATION OF NICOTINE IMPURITIES AND DEGRADANTS IN NICOTINE POUCHES BY LC-MS/MS

(August 2023, Draft Version 3)

0. INTRODUCTION

In 2020, the CORESTA Tobacco and Tobacco Products Analysis (TTPA) Sub-Group conducted a proficiency study for the determination of nicotine and nicotine impurities and degradants (i.e., nicotine degradants) in nicotine pouches.[1,2] Nineteen laboratories participated in the nicotine analysis and fifteen laboratories participated in the nicotine degradants analysis. The objective of this proficiency study was to provide an assessment of laboratory capability for the analysis of nicotine and nicotine degradants in nicotine pouches. Laboratories determined nicotine, anatabine, anabasine, nicotine-N'-oxide, myosmine, β-nicotyrine, cotinine, and nornicotine using their in-house methodologies. The amount of nicotine determined by all labs in all samples was found to be relatively consistent despite the use of various extraction and analytical methods, but still more variable than would be expected with a single standardized method. The proficiency study showed that in many cases the participating laboratories obtained different results for the nicotine degradants which was attributed to the use of different methodologies. Results from this study were discussed at the virtual TTPA meeting held on April 22, 2020 and published in the TTPA-246-CTR technical report.[1] The TTPA Sub-Group recommended that developing a consensus standardized method would likely improve the consistency of the nicotine degradants results. Therefore, a small group was formed to develop a proposal for a draft CORESTA Recommended Method for the determination of nicotine degradants in nicotine pouches.

In 2023, the TTPA Sub-Group completed a collaborative study for the determination of the seven nicotine impurities and degradants in nicotine pouches.[3] The intent of the study was to calculate repeatability and reproducibility values for the method. Four commercial-like nicotine pouch filler samples were included in the study and 8 laboratories participated. This Recommended Method has been shown to be fit for the determination of nicotine impurities and degradants of nicotine pouches.

1. FIELD OF APPLICATION

This recommended method is applicable to the determination of nicotine degradants and impurities in nicotine pouches. Nicotine pouches, or white pouches, are oral tobacco products that contain tobacco derived nicotine, but not tobacco leaf. The nicotine degradants included in this Recommended Method are: anatabine, anabasine, nicotine-N'-oxide, myosmine, β-nicotyrine, cotinine, and nornicotine.

2. NORMATIVE REFERENCES

2.1 ISO 3696, Water for analytical laboratory use - specification and test methods

3. PRINCIPLE

After addition of deuterium isotopically labelled internal standards, the sample is extracted into an aqueous buffer and filtered. The filtrate is analyzed by liquid chromatography - triple quadrupole mass spectrometry (LC-MS/MS). The results are reported in units of micrograms per gram.

4. EQUIPMENT AND APPARATUS

Normal laboratory apparatus and equipment including the following items:

4.1 High performance liquid chromatography coupled to tandem triple quadrupole mass spectrometry (LC-MS/MS) with an electrospray ionization (ESI) source consisting of:

4.1.1 Binary pump

4.1.2 Chilled autosampler

4.1.3 Column oven

4.1.4 Tandem mass spectrometer

4.1.5 Data collection system

4.2 C18 HPLC column, 1,7 μm particle size, 2,1 mm × 100 mm (or 50 mm), or equivalent

4.3 Analytical balance (0,0001 g resolution)

4.4 Orbital shaker, wrist action shaker, or similar

4.5 Amber autosampler vials with PTFE lined caps

4.6 Disposable polypropylene 3 ml syringes

4.7 Syringe filter, 0,2 μm PTFE or equivalent

NOTE: Other filter materials may also be suitable; however, recovery should be evaluated before routine use.

4.8 Amber glass extraction containers, 50 - 100 ml, with PTFE screw cap liners

4.9 Graduated cylinders

4.10 Volumetric flasks, Class A

4.11 Volumetric pipette, calibrated pipette or equivalent

5. REAGENTS

Use reagents of recognized analytical grade and solvents of HPLC-grade or better.

5.1 water, Grade 1 (refer to ISO 3696)

5.2 acetonitrile (HPLC-grade)

5.3 methanol (HPLC-grade)

5.4 Ammonium hydroxide (6 M) (≥ 98 %)

5.5 Ammonium acetate (≥ 98 %)

5.6 Ammonium formate (≥ 98 %)

5.7 Acetic acid (1 M) (≥ 98 %)

5.8 Formic acid (≥ 98 %)

5.9 Anabasine, CAS-No 13078-04-1 (RS) or 494-52-0 (L) (≥ 98 %)

5.10 Anatabine, CAS-No 2743-90-0 (RS) (≥ 95 %)

5.11 Cotinine, CAS-No 486-56-6 (≥ 98 %)

5.12 Nornicotine, CAS-No 5746-86-1 (≥ 94 %)

5.13 Myosmine, CAS-No 532-12-7 (≥ 98 %)

5.14 β-Nicotyrine, CAS-No 487-19-4 (≥ 97 %)

5.15 Nicotine-N'-Oxide, CAS-No 63551-14-4 (≥ 98 %)

5.16 Anabasine-d_4, CAS-No 1020719-08-7 (≥ 98 %)

5.17 Anatabine-d_4, CAS-No 1020719-11-2 (≥ 98 %)

5.18 Cotinine-d_3, CAS-No 110952-70-0 (≥ 98 %)

5.19 Nornicotine-d_4, CAS-No 66148-18-3 (≥ 97 %)

5.20 Myosmine-d_4, CAS-No 66148-17-2 (≥ 98 %)

5.21 β-Nicotyrine-d_3 (≥ 98 %)

5.22 Nicotine-N'-Oxide-d_3 (≥ 98 %)

NOTE: Reference materials should be purchased with high(est) purity available in analytical quality.

6. SOLUTIONS PREPARATION

6.1 Reagents

6.1.1 Extraction Solution

Prepare 2 litres of 100 mM ammonium formate buffer (pH 3) by adding 12,61 g of ammonium formate to a 2000 ml graduated cylinder volumetric flask containing approximately 1800 ml of Grade 1 water. Adjust the pH to 3,0 ± 0,1 with approximately 13 ml formic acid and then dilute to volume using Grade 1 water. Store the solution at room temperature in a 2-l glass bottle.

NOTE: This solution has been shown to be stable for 14 days at room temperature.

6.1.2 Mobile Phase A, 10 mM Ammonium Acetate Buffer

Add approximately 900 ml of Grade 1 water to a 1000-ml graduated cylinder. Add 10 ml of 1 M acetic acid. Add 13,0 ml of 6 M ammonium hydroxide. Adjust the pH to 10,0 ± 0,1 with 6 M ammonium hydroxide or 1 M acetic acid. Dilute to 1000 ml with Grade 1 water. Mix well and store in 1-l glass bottle at room temperature. This solution has been shown to be stable for up to 1 month at room temperature.

6.1.3 0,05 % Ammonium Hydroxide Solution for Calibration Standards

Add 0,2 ml of ammonium hydroxide (25 %) to a 100-ml graduated cylinder. Dilute to 100 ml with Grade 1 water. This solution must be prepared at each time calibration standards are prepared.

6.2 Standards

All standard solutions shall be prepared and stored in amber, or light protected glassware and stored at approximately -20 °C, except the calibration standards shall be stored in a refrigerator. All standards shall be stored in amber glass bottles with PTFE screw caps. Intermediate standard solutions have been shown to be stable for one year. The calibration standards have been shown to be stable for 6 months. Laboratories should determine the stability of the standards under their conditions of use.

NOTE: All solutions shall be prepared using appropriately sized Class A volumetric pipettes and volumetric flasks.

6.2.1 Internal Standard Stock Solutions

Individually weigh approximately 10 mg anatabine-d_4, anabasine-d_4, nicotine-N'-oxide-d_3, myosmine-d_4, β-nicotyrine-d_3, cotinine-d_3, and nornicotine-d_4 into individual 10 ml volumetric flasks. Record the exact weight to 0,01 mg. Dilute to volume with methanol. The concentration in each solution is approximately 1000 µg/ml.

6.2.2 Mixed Internal Standard Solution

Using a Class A volumetric pipette (or calibrated pipette), transfer 1,00 ml of each of the Internal Standard Stock Solutions into a single 10 ml volumetric flask and dilute to volume with methanol, mix well. The concentration of all internal standards is approximately 100 µg/ml.

6.3 Calibration Standard Solutions

6.3.1 Nicotine Degradants Stock Solutions

Individually weigh approximately 10,0 mg anatabine, anabasine, nicotine-N'-oxide, Myosmine, β-nicotyrine, cotinine, and nornicotine into individual 10 ml volumetric flasks. Record the exact weight to 0,01 mg. Dilute to volume with methanol. The concentration in each solution is approximately 1000 µg/ml.

6.3.2 Primary Mixed Nicotine Degradants Standard Solution

Using a Class A volumetric pipette (or calibrated pipette), transfer 1,00 ml of each of the single Nicotine Degradants Stock Solutions into a single 10 ml volumetric flask and dilute to volume with methanol and mix well. The concentration is approximately 100 µg/ml of all seven nicotine degradants.

6.3.3 Secondary Mixed Nicotine Degradants Standard Solution

Using a class A volumetric pipette (or calibrated pipette), transfer 1,00 ml of the Primary Mixed Nicotine Degradants Standard solutions into a single 10 ml volumetric flask and dilute to volume with methanol and mix well. The concentration is approximately 10 µg/ml of all seven nicotine degradants.

6.3.4 Nicotine Degradants Calibration Standards

Prepare 7 working calibration standard solutions that cover the concentration range of interest. An example of calibration standard preparation is provided in Table 1. The nicotine degradants calibration standards are prepared in seven separate 10 ml volumetric flasks. Briefly, add 2 ml of 0,05 % ammonium hydroxide solution to each volumetric flask. Add 20,0 µl of the Mixed Internal Standard Solution (100 µg/ml) to each of the seven volumetric flasks. Next, add the appropriate volume of the primary and secondary nicotine degradants standard solutions specified in Table 1. Finally, each of the seven flasks is diluted to volume with 0,05 % ammonium hydroxide solution and mixed well. Calculate the exact concentrations for each calibration standard.

NOTE: Stock solutions of the individual nicotine degradants and deuterated internal standards in methanol can be purchased at the required concentrations.

NOTE: The linearity range should be determined for each lab/instrument to fit the instruments capabilities and the range of samples usually being measured in that laboratory. Samples can be diluted in 0,5 % ammonium hydroxide solution containing internal standards if necessary.

Table 1. Concentration and preparation of nicotine calibration standards

Calibration Standard	Flask (ml)	Volume of Primary Mixed Nicotine Degradants Standard Solution (100 µg/ml) (µl)	Volume of Secondary Mixed Nicotine Degradants Standard Solution (10 µg/ml) (µl)	Volume of Mixed Internal Standard Solution (100 µg/ml) (µl)	Internal Standard Final Concentration (µg/ml)	Calibration Standard Concentration (µg/ml)
1	10	NA	25	20	0,20	0,025
2	10	NA	100	20	0,20	0,10
3	10	25	NA	20	0,20	0,25
4	10	50	NA	20	0,20	0,50
5	10	100	NA	20	0,20	1,00
6	10	250	NA	20	0,20	2,50
7	10	500	NA	20	0,20	5,00

*NA = Not applicable

7. PROCEDURES

7.1 Sampling

Sampling is conducted such that the laboratory test sample is representative of the population received for analysis.

7.2 Sample Preparation

7.2.1 The target aliquot weight for each replicate analysis is approximately 1,0 gram.

7.2.2 Unit pouches shall be analyzed and include both the paper and filler. Extract a sufficient number of pouches to come as close to the target weight as possible. Cut the pouch into two halves and add the filler and pouch paper directly into the extraction flask.

7.3 Sample Extraction

7.3.1 Using an analytical balance, weigh approximately 1,0 g (note the exact weight with 4 decimals) of sample into the extraction vessel.

7.3.2 Add 60,0 µl of the 100 µg/ml Mixed Internal Standard Solution (using a calibrated pipette or equivalent).

7.3.3 Add 30,0 ml of extraction solution (100 mM ammonium formate (pH 3)) and cap the flask.

7.3.4 Shake vigorously the sample(s) for 40 min ± 5 min at a rate to ensure sufficient mixing and allow samples to settle for 5 minutes then proceed to the next step.

7.3.5 Filter each sample using a 0,2 µm PTFE syringe filter directly into amber vials and cap each vial.

NOTE: Samples may be extracted in a centrifuge tube instead of glass vials and be centrifuged after shaking.

NOTE: Samples that exceed the calibration range may be diluted using the 0,05 % ammonium hydroxide solution.

7.3.6 The extract is ready for injection into the LC-MS/MS system.

NOTE: The stability of the prepared samples in the refrigerator (4–6 ºC) was investigated by analyzing samples immediately after preparation and after 3, 7 and 14 days. The samples were stored in vials with perforated and unperforated septa. The results revealed that the samples were stable for at least seven days in vials with unperforated septa and only three days in vials with perforated septa.

8. DETERMINATION

Set up and operate the LC-MS/MS system in accordance with the manufacturer's instructions. Equilibrate the system prior to use. The instrument conditions given below have been shown to produce suitable results. Depending on the instrument used, it may be necessary to modify the instrument conditions to provide equivalent results.

8.1 Suggested HPLC Parameters

The following are recommended conditions for the LC system and may be modified to achieve acceptable performance:

- Column temperature: 45,0 °C
- Injection volume: 1,0 µl
- Flow rate: 0,4 ml/min
- Autosampler temperature: 10 ºC
- Mobile phase A: 10 mM ammonium acetate, pH 10,0
- Mobile phase B: acetonitrile

Depending on the HPLC column that is used, it may be necessary to adjust the HPLC gradient provided in Table 2.

Table 2. HPLC gradient

Time (min)	Flow (ml/min)	% Eluent A	% Eluent B	Gradient type
Initial	0,40	95	5	Initial
3,0	0,40	45	55	Linear
3,1	0,40	10	90	Linear
4,0	0,40	10	90	Linear
4,5	0,40	95	5	Linear
5,5	0,40	95	5	Linear

8.2 MS/MS Parameters

Setup the triple quadrupole mass spectrometer in positive electrospray mode using multiple reaction monitoring (MRM). It is necessary that the triple quadrupole mass spectrometer has been carefully optimized for sensitivity of each analyte before analysis can occur. The dwell times need to be optimized to achieve accurate quantification and the number of data points across each peak should be 15 to 20. Once optimized, the same LC-MS/MS conditions must be used for the analysis of all standards and samples.

8.2.1 Quantification and Qualification Transitions

The most sensitive ion precursors listed in Table 3 are used for the quantification.

Table 3. Quantification and qualification transitions for nicotine degradants

Analyte	Retention time (min)	Quantification Transition (m/z)	Qualification Transition (m/z)	Internal Standard Reference
Nicotine-N'-Oxide	~1,12	179,0 > 132,1	179,1 > 130	Nicotine-N'-Oxide-d_3
Nicotine-N'-Oxide-d_3	~1,12	182,1 > 130,1	182,1 > 132	
Nornicotine	~1,98	149,1 > 130,1	149,1 > 117; 149,1 > 132	Nornicotine-d_4
Nornicotine-d_4	~1,97	153,1 > 136,1	153,1 > 121	
Cotinine	~1,62	177,0 > 80	177,1 > 98	Cotinine-d_3
Cotinine-d_3	~1,62	180,1 > 80	180,1 > 101	
Anabasine	~2,33	163,1 > 92	163,1 > 146; 163,1 > 94	Anabasine-d_4
Anabasine-d_4	~2,31	167,1 > 96	167,1 > 122	
Anatabine	~2,09	161,1 > 107	161,1 > 144	Anatabine-d_4
Anatabine-d_4	~2,08	165,1 > 148,1	165,1 > 111	
Myosmine	~2,10	147,1 > 105	147,1 > 130	Myosmine-d_4
Myosmine-d_4	~2,09	151,1 > 81	151,1 > 109	
β-Nicotyrine	~2,96	159,1 > 144	159,1 > 117	β-Nicotyrine-d_3
β-Nicotyrine-d_3	~2,95	162,1 > 144	162,1 > 117	

Note: The transitions provided in Table 3 are for guidance purposes only and the actual optimized values may vary from instrument to instrument.

The performance of the system should be sufficient to achieve MRM chromatograms similar to those given in Appendix I.

8.3 System Suitability

The system performance must be evaluated for sensitivity, chromatographic performance, carry over and any other criteria necessary to ensure optimization of the LC-MS/MS system.

8.4 Calibration

Analyse the initial calibration consisting of at least 7 consecutive standard concentrations per analyte. Set the quantitation method to perform an internal standard quadratic calibration with 1/x weighting. The regression should have a coefficient of determination (r^2) of at least 0,995. The calibration curve is a response of the area ratio of each analyte to the corresponding internal standard. Do not force the quadratic correlation through the origin. Inject all calibration standards and then proceed to the samples.

The initial calibration standards are acceptable if they are within 20 % of their assigned values for the lowest standard (LOQ) and within 15 % for all other standards. If the calibration does not meet criteria, check the instrument for problems; analyse fresh standard aliquots and/or fresh standards prepared from stock solutions.

8.5 Calculations

8.5.1 The concentration of the target analyte in a sample (µg/g) is determined using the concentration obtained from the instrument with the following equation:

$$C_{ND} = \left(\frac{C \times V}{W}\right)$$

Where

C_{ND} is the nicotine degradants concentration in µg/g
C is the nicotine degradants concentration obtained from calibration curve in µg/ml
V is the extraction volume in ml

W is the sample weight in g

8.5.2 The concentration of the target analyte in a sample (µg/portion) is determined using the calculated µg/g concentration with the following equation:

$$C_P = C_{ND} \text{ x } N$$

Where

C_P is the nicotine degradants concentration in µg/portion
C_{ND} is the nicotine degradants concentration in µg/g
N is the portion weight in g

8.6 Quality Control

Each laboratory should perform quality control procedures per their quality system requirements.

9. SUGGESTED SPECIAL PRECAUTIONS

9.1 Experience has shown that the nicotine pouch matrix might lead to contamination of the ion source resulting in poor response and elevated background noise. One way to decrease contamination of the ion source is to use a switch between the column and the ion source to divert the flow prior to the analytes eluting from the column. A guard column might also be used to decrease contaminations.

10. REPEATABILITY AND REPRODUCIBILITY

[To be added after completion of the Collaborative Study]

11. TEST REPORT

The test report shall state the amount of analyte per gram and per portion (as received or 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 the test samples.

Moisture content may be determined on separate sample aliquots if it is necessary to present the 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 [3].

12. BIBLIOGRAPHY

[1] TTPA-246, Technical Report, 2020 Nicotine and nicotine degradants proficiency study, September 2020.

[2] Avagyan, R.; Spasova, M.; Lindholm, J. Determination of Nicotine-Related Impurities in Nicotine Pouches and Tobacco-Containing Products by Liquid Chromatography–Tandem Mass Spectrometry. *Separations* 2021, *8*, 77. https://doi.org/10.3390/separations8060077

[3] TTPA-246, Technical Report, 2023 Collaborative Study for the determination of Nicotine Impurities and degradants in Nicotine Pouches by LC-MS, [To be added after Collaborative Study].

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

APPENDIX I – Example Chromatograms

Figure 1 - Example of a MRM-chromatogram for a nicotine degradants standard (5 µg/ml)

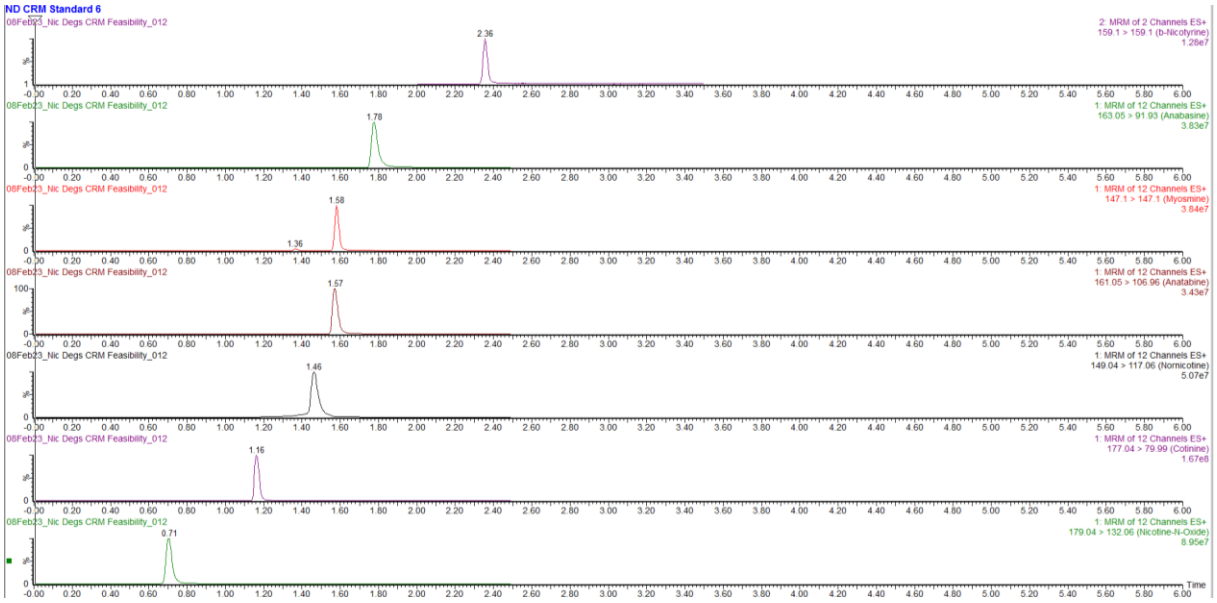
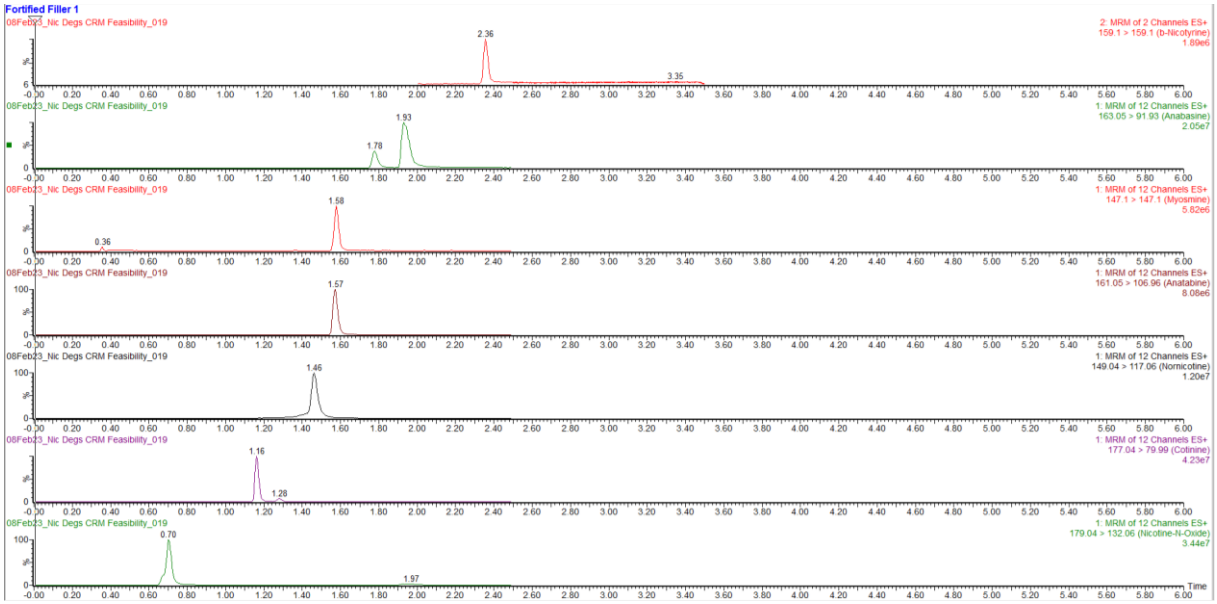


Figure 2 - Example of a MRM-chromatogram for a nicotine pouch sample



APPENDIX C: Full Data Set

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

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
1	Day 1	Filler-1	1	ND	<LOQ	<LOQ	1,126	<LOQ	11,464	2,311
1	Day 1	Filler-1	2	ND	<LOQ	<LOQ	1,050	<LOQ	11,343	2,221
1	Day 1	Filler-1	3	ND	<LOQ	<LOQ	1,105	<LOQ	11,138	2,359
1	Day 1	Filler-2	1	ND	ND	0,835	3,639	2,088	442,975	13,453
1	Day 1	Filler-2	2	ND	ND	0,726	3,752	2,118	445,991	12,859
1	Day 1	Filler-2	3	ND	ND	0,793	3,731	2,086	454,216	13,632
1	Day 1	Filler-3	1	ND	<LOQ	1,077	2,303	1,047	56,745	11,606
1	Day 1	Filler-3	2	ND	<LOQ	0,925	2,298	0,985	55,756	11,103
1	Day 1	Filler-3	3	ND	<LOQ	0,982	2,292	1,012	55,999	10,569
1	Day 1	Filler-4	1	ND	ND	<LOQ	6,767	1,448	372,636	6,117
1	Day 1	Filler-4	2	ND	ND	<LOQ	6,678	1,527	373,428	6,229
1	Day 1	Filler-4	3	ND	ND	<LOQ	6,709	1,498	370,807	5,960
1	Day 1	Fortified Filler-1	1	10,539	11,198	10,898	11,527	10,988	21,377	12,874
1	Day 1	Fortified Filler-1	2	10,203	10,442	11,008	11,426	10,889	21,092	12,470
1	Day 1	Fortified Filler-1	3	10,217	10,934	10,844	11,860	11,024	21,330	12,727
1	Day 2	Filler-1	1	ND	<LOQ	<LOQ	1,070	<LOQ	11,265	2,348
1	Day 2	Filler-1	2	ND	<LOQ	<LOQ	1,050	<LOQ	11,079	2,274
1	Day 2	Filler-1	3	ND	<LOQ	<LOQ	1,078	<LOQ	11,079	2,366

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
1	Day 2	Filler-2	1	ND	ND	<LOQ	3,978	2,123	473,731	13,907
1	Day 2	Filler-2	2	ND	ND	<LOQ	3,983	2,154	473,793	12,833
1	Day 2	Filler-2	3	ND	ND	<LOQ	3,922	2,096	460,180	13,054
1	Day 2	Filler-3	1	ND	<LOQ	1,005	2,365	1,123	56,039	10,788
1	Day 2	Filler-3	2	ND	<LOQ	1,065	2,307	1,035	57,250	10,557
1	Day 2	Filler-3	3	ND	<LOQ	0,997	2,346	1,056	55,401	10,734
1	Day 2	Filler-4	1	ND	ND	<LOQ	6,911	1,370	376,825	5,719
1	Day 2	Filler-4	2	ND	ND	<LOQ	6,896	1,438	382,923	5,575
1	Day 2	Filler-4	3	ND	ND	<LOQ	6,941	1,406	375,785	5,415
1	Day 2	Fortified Filler-1	1	10,036	10,736	10,736	11,553	10,911	21,793	12,662
1	Day 2	Fortified Filler-1	2	10,039	10,696	9,979	11,742	11,383	21,542	12,549
1	Day 2	Fortified Filler-1	3	9,798	10,715	10,656	11,663	10,715	21,431	12,047
1	Day 3	Filler-1	1	ND	<LOQ	<LOQ	1,127	<LOQ	11,957	2,403
1	Day 3	Filler-1	2	ND	<LOQ	ND	1,133	<LOQ	11,689	2,445
1	Day 3	Filler-1	3	ND	<LOQ	<LOQ	1,167	<LOQ	11,398	2,453
1	Day 3	Filler-2	1	ND	ND	0,804	4,198	2,084	459,065	14,826
1	Day 3	Filler-2	2	ND	ND	0,868	4,190	2,095	459,352	14,155
1	Day 3	Filler-2	3	ND	ND	0,807	4,007	2,153	461,975	14,113
1	Day 3	Filler-3	1	ND	<LOQ	1,045	2,389	1,045	55,938	11,259
1	Day 3	Filler-3	2	ND	<LOQ	0,915	2,479	1,121	56,931	12,189

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
1	Day 3	Filler-3	3	ND	<LOQ	0,932	2,388	1,107	56,534	11,214
1	Day 3	Filler-4	1	ND	ND	<LOQ	7,014	1,433	365,599	5,730
1	Day 3	Filler-4	2	ND	ND	<LOQ	6,767	1,412	375,699	5,737
1	Day 3	Filler-4	3	ND	ND	<LOQ	6,898	1,385	369,068	5,778
1	Day 3	Fortified Filler-1	1	9,949	10,766	9,833	11,437	10,708	21,008	12,050
1	Day 3	Fortified Filler-1	2	10,068	10,924	10,334	11,515	11,013	21,169	13,050
1	Day 3	Fortified Filler-1	3	10,161	11,114	10,191	11,681	10,965	21,603	13,200
1	Day 4	Filler-1	1	ND	<LOQ	<LOQ	1,098	<LOQ	11,451	2,344
1	Day 4	Filler-1	2	ND	<LOQ	<LOQ	1,126	<LOQ	11,499	2,460
1	Day 4	Filler-1	3	ND	<LOQ	<LOQ	1,274	<LOQ	12,678	2,844
1	Day 4	Filler-2	1	ND	ND	0,780	4,348	2,159	515,142	15,082
1	Day 4	Filler-2	2	ND	ND	0,715	4,169	2,144	516,625	15,305
1	Day 4	Filler-2	3	ND	ND	0,777	4,216	2,093	495,166	14,801
1	Day 4	Filler-3	1	ND	<LOQ	1,012	2,500	1,220	58,274	11,220
1	Day 4	Filler-3	2	ND	<LOQ	1,015	2,417	1,134	57,201	11,458
1	Day 4	Filler-3	3	ND	<LOQ	1,105	2,419	1,135	57,820	10,871
1	Day 4	Filler-4	1	ND	ND	<LOQ	6,929	1,493	402,887	5,734
1	Day 4	Filler-4	2	ND	ND	<LOQ	7,108	1,463	400,796	5,913
1	Day 4	Filler-4	3	ND	ND	<LOQ	7,020	1,404	395,798	5,944
1	Day 4	Fortified Filler-1	1	9,587	10,246	10,306	11,084	10,336	21,330	13,122

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
1	Day 4	Fortified Filler-1	2	10,264	10,948	10,561	11,900	11,156	21,956	13,655
1	Day 4	Fortified Filler-1	3	10,952	11,101	10,952	12,173	11,488	22,054	13,780
2	Day 1	Filler-1	1	<LOQ	<LOQ	<LOQ	1,35	<LOQ	11,15	2,54
2	Day 1	Filler-1	2	<LOQ	<LOQ	<LOQ	1,35	<LOQ	11,17	2,45
2	Day 1	Filler-1	3	<LOQ	<LOQ	<LOQ	1,34	<LOQ	10,74	2,52
2	Day 1	Filler-2	1	<LOQ	<LOQ	1,10	3,75	1,87	449,60	10,74
2	Day 1	Filler-2	2	<LOQ	<LOQ	1,15	3,73	1,85	442,56	10,70
2	Day 1	Filler-2	3	<LOQ	<LOQ	1,12	3,73	1,83	442,01	10,60
2	Day 1	Filler-3	1	0,78	<LOQ	1,25	2,42	1,30	57,21	10,58
2	Day 1	Filler-3	2	0,82	<LOQ	1,24	2,38	1,19	55,02	10,30
2	Day 1	Filler-3	3	0,79	<LOQ	1,22	2,36	1,23	54,02	9,90
2	Day 1	Filler-4	1	<LOQ	<LOQ	<LOQ	6,69	1,21	361,66	4,95
2	Day 1	Filler-4	2	<LOQ	<LOQ	<LOQ	6,55	1,19	364,09	5,04
2	Day 1	Filler-4	3	<LOQ	<LOQ	<LOQ	6,67	1,24	376,84	5,02
2	Day 1	Fortified Filler-1	1	9,24	8,29	9,46	10,28	9,49	20,97	10,92
2	Day 1	Fortified Filler-1	2	9,63	8,74	9,93	10,62	9,62	19,92	11,38
2	Day 1	Fortified Filler-1	3	9,84	8,77	10,61	10,75	9,36	21,04	11,36
2	Day 2	Filler-1	1	<LOQ	<LOQ	<LOQ	1,47	<LOQ	10,68	2,48
2	Day 2	Filler-1	2	<LOQ	<LOQ	<LOQ	1,46	<LOQ	11,47	2,53

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
2	Day 2	Filler-1	3	<LOQ	<LOQ	<LOQ	1,50	<LOQ	11,12	2,51
2	Day 2	Filler-2	1	<LOQ	<LOQ	1,07	3,67	1,90	405,83	10,87
2	Day 2	Filler-2	2	<LOQ	<LOQ	1,05	3,74	2,04	396,36	11,00
2	Day 2	Filler-2	3	<LOQ	<LOQ	1,06	3,51	2,06	396,43	10,85
2	Day 2	Filler-3	1	0,78	<LOQ	1,16	2,38	1,27	50,73	10,84
2	Day 2	Filler-3	2	0,78	<LOQ	1,22	2,41	1,34	53,48	10,76
2	Day 2	Filler-3	3	0,77	<LOQ	1,20	2,38	1,34	53,44	10,64
2	Day 2	Filler-4	1	<LOQ	<LOQ	<0,75	6,34	1,37	335,82	5,21
2	Day 2	Filler-4	2	<LOQ	<LOQ	<0,75	6,25	1,42	324,77	5,24
2	Day 2	Filler-4	3	<LOQ	<LOQ	<0,75	6,24	1,36	377,22	4,94
2	Day 2	Fortified Filler-1	1	9,70	9,25	9,64	10,38	9,97	19,91	11,73
2	Day 2	Fortified Filler-1	2	9,50	9,72	9,46	10,36	10,07	20,41	12,16
2	Day 2	Fortified Filler-1	3	9,53	9,52	9,64	10,66	10,16	19,87	12,19
2	Day 3	Filler-1	1	<LOQ	<LOQ	<LOQ	1,34	<LOQ	10,74	2,52
2	Day 3	Filler-1	2	<LOQ	<LOQ	<LOQ	1,38	<LOQ	10,47	2,46
2	Day 3	Filler-1	3	<LOQ	<LOQ	<LOQ	1,43	<LOQ	10,41	2,73
2	Day 3	Filler-2	1	<LOQ	<LOQ	1,02	3,77	1,98	384,87	10,36
2	Day 3	Filler-2	2	<LOQ	<LOQ	1,00	3,65	1,82	390,53	10,03
2	Day 3	Filler-2	3	<LOQ	<LOQ	1,08	3,63	2,13	409,55	10,23
2	Day 3	Filler-3	1	0,76	<LOQ	1,32	2,37	1,16	48,54	10,15

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
2	Day 3	Filler-3	2	0,76	<LOQ	1,20	2,36	1,15	49,62	9,90
2	Day 3	Filler-3	3	0,76	<LOQ	1,14	2,28	1,22	50,52	9,86
2	Day 3	Filler-4	1	<LOQ	<LOQ	<LOQ	6,30	1,16	345,24	4,98
2	Day 3	Filler-4	2	<LOQ	<LOQ	<LOQ	6,49	1,21	350,55	5,02
2	Day 3	Filler-4	3	<LOQ	<LOQ	<LOQ	6,27	1,27	342,33	4,98
2	Day 3	Fortified Filler-1	1	10,09	9,95	10,79	10,87	10,14	19,71	11,96
2	Day 3	Fortified Filler-1	2	10,21	9,79	10,19	10,71	10,40	19,16	11,71
2	Day 3	Fortified Filler-1	3	10,19	9,79	10,79	11,15	10,78	19,23	11,86
2	Day 4	Filler-1	1	<LOQ	<LOQ	<LOQ	1,45	<LOQ	10,68	2,19
2	Day 4	Filler-1	2	<LOQ	<LOQ	<LOQ	1,54	<LOQ	10,63	2,26
2	Day 4	Filler-1	3	<LOQ	<LOQ	<LOQ	1,53	<LOQ	11,12	2,28
2	Day 4	Filler-2	1	<LOQ	<LOQ	1,04	3,73	1,97	427,21	10,42
2	Day 4	Filler-2	2	<LOQ	<LOQ	0,99	3,71	2,12	421,78	10,75
2	Day 4	Filler-2	3	<LOQ	<LOQ	0,98	3,86	2,13	425,05	10,79
2	Day 4	Filler-3	1	0,85	<LOQ	1,13	2,65	1,34	52,08	10,53
2	Day 4	Filler-3	2	0,85	<LOQ	1,12	2,72	1,31	51,88	10,40
2	Day 4	Filler-3	3	0,84	<LOQ	1,18	2,63	1,31	54,22	10,49
2	Day 4	Filler-4	1	<LOQ	<LOQ	<0,75	6,46	1,23	368,24	4,98
2	Day 4	Filler-4	2	<LOQ	<LOQ	<0,75	6,49	1,31	361,87	5,22
2	Day 4	Filler-4	3	<LOQ	<LOQ	<0,75	6,43	1,39	353,31	5,13

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
2	Day 4	Fortified Filler-1	1	9,03	10,13	10,49	11,50	10,39	21,12	11,80
2	Day 4	Fortified Filler-1	2	9,17	10,24	10,70	11,42	9,93	19,95	12,00
2	Day 4	Fortified Filler-1	3	9,02	10,15	10,86	11,35	10,14	20,41	11,72
3	Day 1	Filler-1	1	<LOQ	<LOQ	<LOQ	1,202	<LOQ	9,244	2,898
3	Day 1	Filler-1	2	<LOQ	<LOQ	<LOQ	1,188	<LOQ	8,852	2,841
3	Day 1	Filler-1	3	<LOQ	<LOQ	<LOQ	1,199	<LOQ	9,006	2,783
3	Day 1	Filler-2	1	<LOQ	<LOQ	0,885	3,343	2,118	432,670	14,304
3	Day 1	Filler-2	2	<LOQ	<LOQ	0,884	3,448	2,066	428,998	14,710
3	Day 1	Filler-2	3	<LOQ	<LOQ	0,917	3,464	2,137	423,758	14,105
3	Day 1	Filler-3	1	<LOQ	<LOQ	1,082	2,079	1,350	37,110	13,599
3	Day 1	Filler-3	2	<LOQ	<LOQ	1,035	2,014	1,334	35,636	13,242
3	Day 1	Filler-3	3	<LOQ	<LOQ	1,119	2,032	1,283	36,366	13,666
3	Day 1	Filler-4	1	<LOQ	<LOQ	<LOQ	5,937	1,521	344,769	6,438
3	Day 1	Filler-4	2	<LOQ	<LOQ	<LOQ	5,858	1,531	340,152	6,332
3	Day 1	Filler-4	3	<LOQ	<LOQ	<LOQ	5,869	1,498	343,816	6,723
3	Day 1	Fortified Filler-1	1	9,195	11,178	10,501	11,599	11,000	11,301	16,069
3	Day 1	Fortified Filler-1	2	8,977	11,823	10,742	12,034	11,271	28,608	16,195
3	Day 1	Fortified Filler-1	3	17,203	11,189	11,289	12,233	11,482	33,693	15,268

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
3	Day 2	Filler-1	1	<LOQ	<LOQ	<LOQ	1,214	<LOQ	9,906	2,540
3	Day 2	Filler-1	2	<LOQ	<LOQ	<LOQ	1,199	<LOQ	9,538	2,383
3	Day 2	Filler-1	3	<LOQ	<LOQ	<LOQ	1,176	<LOQ	9,651	2,357
3	Day 2	Filler-2	1	<LOQ	<LOQ	1,011	3,546	2,113	395,643	12,512
3	Day 2	Filler-2	2	<LOQ	<LOQ	0,992	3,505	2,126	394,963	11,877
3	Day 2	Filler-2	3	<LOQ	<LOQ	1,013	3,546	2,109	404,699	12,309
3	Day 2	Filler-3	1	<LOQ	<LOQ	1,180	1,738	1,265	32,914	11,198
3	Day 2	Filler-3	2	<LOQ	<LOQ	1,137	1,734	1,313	32,636	10,406
3	Day 2	Filler-3	3	<LOQ	<LOQ	1,159	1,738	1,401	32,852	11,669
3	Day 2	Filler-4	1	<LOQ	<LOQ	<LOQ	5,884	1,583	322,518	4,983
3	Day 2	Filler-4	2	<LOQ	<LOQ	<LOQ	5,894	1,512	321,268	5,213
3	Day 2	Filler-4	3	<LOQ	<LOQ	<LOQ	5,860	1,484	316,016	5,528
3	Day 2	Fortified Filler-1	1	9,595	10,287	10,097	10,975	10,606	24,046	1,340
3	Day 2	Fortified Filler-1	2	9,408	10,364	9,757	11,074	10,681	24,603	1,233
3	Day 2	Fortified Filler-1	3	9,367	10,679	9,898	10,969	10,348	24,557	1,311
3	Day 3	Filler-1	1	<LOQ	<LOQ	<LOQ	1,251	<LOQ	7,079	4,029
3	Day 3	Filler-1	2	<LOQ	<LOQ	<LOQ	1,190	<LOQ	6,875	3,894
3	Day 3	Filler-1	3	<LOQ	<LOQ	<LOQ	1,179	<LOQ	6,455	4,107
3	Day 3	Filler-2	1	<LOQ	<LOQ	1,053	3,314	2,146	297,214	16,059
3	Day 3	Filler-2	2	<LOQ	<LOQ	0,925	3,354	2,149	304,875	15,168

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
3	Day 3	Filler-2	3	<LOQ	<LOQ	0,854	3,462	2,236	298,991	15,477
3	Day 3	Filler-3	1	<LOQ	<LOQ	1,257	2,020	1,443	32,006	16,296
3	Day 3	Filler-3	2	<LOQ	<LOQ	1,080	2,045	1,331	32,287	15,540
3	Day 3	Filler-3	3	<LOQ	<LOQ	1,222	2,029	1,353	31,343	16,229
3	Day 3	Filler-4	1	<LOQ	<LOQ	<LOQ	5,854	1,555	247,378	9,775
3	Day 3	Filler-4	2	<LOQ	<LOQ	<LOQ	5,914	1,632	250,094	9,784
3	Day 3	Filler-4	3	<LOQ	<LOQ	<LOQ	5,850	1,579	248,803	9,571
3	Day 3	Fortified Filler-1	1	10,702	11,801	10,944	12,258	11,980	16,621	1,560
3	Day 3	Fortified Filler-1	2	9,387	10,131	9,742	10,745	10,535	23,357	1,123
3	Day 3	Fortified Filler-1	3	9,946	11,330	10,561	11,675	11,002	26,270	1,711
3	Day 4	Filler-1	1	<LOQ	<LOQ	<LOQ	1,272	<LOQ	6,262	5,414
3	Day 4	Filler-1	2	<LOQ	<LOQ	<LOQ	1,249	<LOQ	6,278	4,066
3	Day 4	Filler-1	3	<LOQ	<LOQ	<LOQ	1,294	<LOQ	6,446	4,131
3	Day 4	Filler-2	1	<LOQ	<LOQ	0,915	3,599	2,147	311,371	14,774
3	Day 4	Filler-2	2	<LOQ	<LOQ	0,923	3,616	2,122	306,422	15,287
3	Day 4	Filler-2	3	<LOQ	<LOQ	0,883	3,625	2,201	306,065	14,337
3	Day 4	Filler-3	1	<LOQ	<LOQ	1,227	2,153	1,271	27,180	14,456
3	Day 4	Filler-3	2	<LOQ	<LOQ	1,162	2,254	1,293	28,565	14,823
3	Day 4	Filler-3	3	<LOQ	<LOQ	1,115	2,275	1,341	29,205	14,854
3	Day 4	Filler-4	1	<LOQ	<LOQ	<LOQ	6,195	1,639	264,530	9,946

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
3	Day 4	Filler-4	2	<LOQ	<LOQ	<LOQ	5,998	1,526	264,572	9,921
3	Day 4	Filler-4	3	<LOQ	<LOQ	<LOQ	6,148	1,504	272,399	10,071
3	Day 4	Fortified Filler-1	1	7,883	8,840	8,369	9,388	8,591	12,863	1,354
3	Day 4	Fortified Filler-1	2	10,608	11,802	11,230	12,413	11,306	24,097	1,713
3	Day 4	Fortified Filler-1	3	10,213	11,322	10,593	11,882	11,338	23,374	1,579
4	Day 1	Filler-1	1	<LOQ	<LOQ	<LOQ	1,112	<LOQ	10,877	4,271
4	Day 1	Filler-1	2	<LOQ	<LOQ	<LOQ	1,158	<LOQ	10,392	4,458
4	Day 1	Filler-1	3	<LOQ	<LOQ	<LOQ	1,090	<LOQ	11,198	4,172
4	Day 1	Filler-2	1	<LOQ	<LOQ	0,878	3,403	1,856	405,450	13,617
4	Day 1	Filler-2	2	<LOQ	<LOQ	0,776	3,477	1,842	433,240	12,835
4	Day 1	Filler-2	3	<LOQ	<LOQ	0,985	3,629	1,888	421,448	12,523
4	Day 1	Filler-3	1	<LOQ	<LOQ	0,986	2,537	1,012	53,198	13,905
4	Day 1	Filler-3	2	<LOQ	<LOQ	1,066	2,517	1,009	53,454	13,337
4	Day 1	Filler-3	3	<LOQ	<LOQ	0,990	2,238	0,997	52,339	13,105
4	Day 1	Filler-4	1	<LOQ	<LOQ	<LOQ	6,718	1,340	347,440	8,518
4	Day 1	Filler-4	2	<LOQ	<LOQ	<LOQ	6,425	1,339	353,455	9,66
4	Day 1	Filler-4	3	<LOQ	<LOQ	<LOQ	6,250	1,320	348,891	9,78
4	Day 1	Fortified Filler-1	1	9,491	10,281	9,156	10,205	9,609	19,158	13,884
4	Day 1	Fortified Filler-1	2	9,201	9,811	9,716	11,284	10,056	19,443	13,383

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
4	Day 1	Fortified Filler-1	3	9,858	10,651	9,363	10,793	9,871	20,139	13,090
4	Day 2	Filler-1	1	<LOQ	<LOQ	<LOQ	1,105	<LOQ	10,743	3,979
4	Day 2	Filler-1	2	<LOQ	<LOQ	<LOQ	1,131	<LOQ	10,943	3,792
4	Day 2	Filler-1	3	<LOQ	<LOQ	<LOQ	1,165	<LOQ	10,561	3,684
4	Day 2	Filler-2	1	<LOQ	<LOQ	0,757	3,711	1,890	424,967	12,995
4	Day 2	Filler-2	2	<LOQ	<LOQ	0,756	3,536	1,986	441,923	13,525
4	Day 2	Filler-2	3	<LOQ	<LOQ	0,835	3,706	1,880	414,481	13,405
4	Day 2	Filler-3	1	<LOQ	<LOQ	0,996	2,366	0,927	54,146	12,745
4	Day 2	Filler-3	2	<LOQ	<LOQ	0,944	2,321	0,951	54,710	12,681
4	Day 2	Filler-3	3	<LOQ	<LOQ	1,054	2,412	1,023	53,554	12,320
4	Day 2	Filler-4	1	<LOQ	<LOQ	<LOQ	6,289	1,225	373,925	8,193
4	Day 2	Filler-4	2	<LOQ	<LOQ	<LOQ	6,537	1,173	380,699	8,186
4	Day 2	Filler-4	3	<LOQ	<LOQ	<LOQ	6,749	1,238	353,745	8,639
4	Day 2	Fortified Filler-1	1	10,711	11,220	9,860	11,171	10,489	20,395	12,186
4	Day 2	Fortified Filler-1	2	10,801	10,780	9,607	11,463	10,360	20,266	13,667
4	Day 2	Fortified Filler-1	3	10,222	10,017	9,587	11,429	10,497	20,353	13,375
4	Day 3	Filler-1	1	<LOQ	<LOQ	<LOQ	0,991	<LOQ	10,781	4,161
4	Day 3	Filler-1	2	<LOQ	<LOQ	<LOQ	1,028	<LOQ	11,289	3,561
4	Day 3	Filler-1	3	<LOQ	<LOQ	<LOQ	1,119	<LOQ	10,712	3,998
4	Day 3	Filler-2	1	<LOQ	<LOQ	<LOQ	3,458	1,996	441,444	13,401

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
4	Day 3	Filler-2	2	<LOQ	<LOQ	<LOQ	3,438	1,908	440,018	13,321
4	Day 3	Filler-2	3	<LOQ	<LOQ	<LOQ	3,399	1,857	417,136	13,915
4	Day 3	Filler-3	1	<LOQ	<LOQ	0,986	2,307	0,968	53,085	12,376
4	Day 3	Filler-3	2	<LOQ	<LOQ	0,95	2,337	0,970	55,176	13,423
4	Day 3	Filler-3	3	<LOQ	<LOQ	1,005	2,262	1,075	55,559	13,628
4	Day 3	Filler-4	1	<LOQ	<LOQ	<LOQ	6,081	1,259	350,952	8,190
4	Day 3	Filler-4	2	<LOQ	<LOQ	<LOQ	6,69	1,337	344,036	8,612
4	Day 3	Filler-4	3	<LOQ	<LOQ	<LOQ	6,067	1,299	348,220	9,154
4	Day 3	Fortified Filler-1	1	9,516	9,980	10,024	10,446	10,657	21,835	13,274
4	Day 3	Fortified Filler-1	2	10,257	10,276	10,066	10,217	10,511	20,653	13,593
4	Day 3	Fortified Filler-1	3	10,918	11,597	10,872	11,006	10,677	21,534	13,247
4	Day 4	Filler-1	1	<LOQ	<LOQ	<LOQ	1,125	<LOQ	10,916	3,652
4	Day 4	Filler-1	2	<LOQ	<LOQ	<LOQ	1,048	<LOQ	10,829	3,489
4	Day 4	Filler-1	3	<LOQ	<LOQ	<LOQ	1,116	<LOQ	11,634	3,592
4	Day 4	Filler-2	1	<LOQ	<LOQ	<LOQ	3,786	1,913	421,698	12,934
4	Day 4	Filler-2	2	<LOQ	<LOQ	<LOQ	3,741	1,876	422,080	13,353
4	Day 4	Filler-2	3	<LOQ	<LOQ	<LOQ	3,738	1,733	414,451	12,338
4	Day 4	Filler-3	1	<LOQ	<LOQ	0,848	2,301	0,927	52,471	11,706
4	Day 4	Filler-3	2	<LOQ	<LOQ	0,928	2,370	1,026	52,869	12,389
4	Day 4	Filler-3	3	<LOQ	<LOQ	0,888	2,458	0,961	53,263	12,699

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
4	Day 4	Filler-4	1	<LOQ	<LOQ	<LOQ	6,692	1,324	324,243	8,781
4	Day 4	Filler-4	2	<LOQ	<LOQ	<LOQ	6,699	1,242	335,681	9,208
4	Day 4	Filler-4	3	<LOQ	<LOQ	<LOQ	6,15	1,237	354,518	9,336
4	Day 4	Fortified Filler-1	1	10,833	10,776	9,717	11,820	9,993	21,084	12,673
4	Day 4	Fortified Filler-1	2	10,446	10,130	9,848	11,232	10,157	20,813	12,790
4	Day 4	Fortified Filler-1	3	9,849	11,034	10,359	10,825	10,019	20,918	13,346
5	Day 1	Filler-1	1	<LOQ	<LOQ	<LOQ	1,409	<LOQ	9,217	2,480
5	Day 1	Filler-1	2	<LOQ	<LOQ	<LOQ	1,211	<LOQ	8,287	2,247
5	Day 1	Filler-1	3	<LOQ	<LOQ	<LOQ	1,196	<LOQ	8,273	2,152
5	Day 1	Filler-2	1	<LOQ	<LOQ	1,109	3,585	2,098	340,162	11,030
5	Day 1	Filler-2	2	<LOQ	<LOQ	1,182	3,609	2,213	341,390	11,308
5	Day 1	Filler-2	3	<LOQ	<LOQ	1,226	3,685	2,060	345,794	11,218
5	Day 1	Filler-3	1	<LOQ	<LOQ	NA	2,206	1,329	42,849	9,378
5	Day 1	Filler-3	2	<LOQ	<LOQ	NA	2,364	1,171	43,628	9,270
5	Day 1	Filler-3	3	<LOQ	<LOQ	NA	2,284	1,652	44,020	8,783
5	Day 1	Filler-4	1	<LOQ	<LOQ	<LOQ	6,347	1,365	307,732	4,499
5	Day 1	Filler-4	2	<LOQ	<LOQ	<LOQ	6,237	1,465	293,690	5,165
5	Day 1	Filler-4	3	<LOQ	<LOQ	<LOQ	6,268	1,480	285,338	5,490
5	Day 1	Fortified Filler-1	1	10,112	10,162	8,353	10,339	10,093	17,060	11,715

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
5	Day 1	Fortified Filler-1	2	10,106	9,999	8,025	10,545	10,220	17,197	12,068
5	Day 1	Fortified Filler-1	3	10,141	10,098	8,100	10,785	10,725	17,553	12,370
5	Day 2	Filler-1	1	<LOQ	<LOQ	<LOQ	1,011	<LOQ	9,069	1,984
5	Day 2	Filler-1	2	<LOQ	<LOQ	<LOQ	1,058	<LOQ	9,126	1,946
5	Day 2	Filler-1	3	<LOQ	<LOQ	<LOQ	1,005	<LOQ	9,004	2,031
5	Day 2	Filler-2	1	<LOQ	<LOQ	<LOQ	3,394	1,908	334,168	10,478
5	Day 2	Filler-2	2	<LOQ	<LOQ	<LOQ	3,449	2,023	343,612	10,608
5	Day 2	Filler-2	3	<LOQ	<LOQ	<LOQ	3,606	2,077	354,464	10,905
5	Day 2	Filler-3	1	<LOQ	<LOQ	NA	2,042	1,127	46,555	10,063
5	Day 2	Filler-3	2	<LOQ	<LOQ	NA	1,692	1,068	39,394	7,945
5	Day 2	Filler-3	3	<LOQ	<LOQ	NA	2,300	1,925	50,892	8,900
5	Day 2	Filler-4	1	<LOQ	<LOQ	<LOQ	6,241	1,450	291,155	4,826
5	Day 2	Filler-4	2	<LOQ	<LOQ	<LOQ	6,271	1,306	291,928	4,949
5	Day 2	Filler-4	3	<LOQ	<LOQ	<LOQ	6,489	1,500	295,412	5,331
5	Day 2	Fortified Filler-1	1	10,112	10,162	8,353	10,339	10,093	17,060	11,715
5	Day 2	Fortified Filler-1	2	10,106	9,999	8,025	10,545	10,220	17,197	12,068
5	Day 2	Fortified Filler-1	3	10,141	10,098	8,100	10,785	10,725	17,553	12,370
5	Day 3	Filler-1	1	<LOQ	<LOQ	<LOQ	1,050	<LOQ	8,522	2,002
5	Day 3	Filler-1	2	<LOQ	<LOQ	<LOQ	0,979	<LOQ	8,307	1,966

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
5	Day 3	Filler-1	3	<LOQ	<LOQ	<LOQ	1,043	<LOQ	8,693	1,990
5	Day 3	Filler-2	1	<LOQ	<LOQ	<LOQ	3,394	1,908	334,168	10,478
5	Day 3	Filler-2	2	<LOQ	<LOQ	<LOQ	3,449	2,023	343,612	10,608
5	Day 3	Filler-2	3	<LOQ	<LOQ	<LOQ	3,606	2,077	354,464	10,905
5	Day 3	Filler-3	1	<LOQ	<LOQ	NA	2,054	1,281	44,685	8,742
5	Day 3	Filler-3	2	<LOQ	<LOQ	NA	1,997	1,075	44,614	10,303
5	Day 3	Filler-3	3	<LOQ	<LOQ	NA	2,109	1,030	44,768	8,595
5	Day 3	Filler-4	1	<LOQ	<LOQ	<LOQ	6,180	1,450	270,812	4,937
5	Day 3	Filler-4	2	<LOQ	<LOQ	<LOQ	6,156	1,208	264,130	5,017
5	Day 3	Filler-4	3	<LOQ	<LOQ	<LOQ	6,168	1,348	269,543	5,168
5	Day 3	Fortified Filler-1	1	10,608	10,185	8,418	10,966	11,652	18,807	12,077
5	Day 3	Fortified Filler-1	2	11,066	10,326	7,969	11,238	11,281	19,278	12,169
5	Day 3	Fortified Filler-1	3	10,880	10,112	7,503	11,138	11,003	18,841	12,020
5	Day 4	Filler-1	1	<LOQ	<LOQ	<LOQ	0,962	<0,750	8,131	1,954
5	Day 4	Filler-1	2	<LOQ	<LOQ	<LOQ	0,991	<0,750	8,272	1,942
5	Day 4	Filler-1	3	NA	NA	NA	NA	NA	NA	NA
5	Day 4	Filler-2	1	<LOQ	<LOQ	<LOQ	2,055	1,311	307,423	6,624
5	Day 4	Filler-2	2	<LOQ	<LOQ	<LOQ	2,063	1,186	309,314	6,422
5	Day 4	Filler-2	3	<LOQ	<LOQ	<LOQ	2,019	1,290	301,540	6,705
5	Day 4	Filler-3	1	<LOQ	<LOQ	NA	2,020	1,003	43,911	8,969

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
5	Day 4	Filler-3	2	<LOQ	<LOQ	NA	1,995	1,186	41,968	9,556
5	Day 4	Filler-3	3	<LOQ	<LOQ	NA	2,065	1,451	42,993	9,744
5	Day 4	Filler-4	1	<LOQ	<LOQ	<LOQ	6,085	1,479	291,219	4,628
5	Day 4	Filler-4	2	<LOQ	<LOQ	<LOQ	6,137	1,328	293,174	4,985
5	Day 4	Filler-4	3	<LOQ	<LOQ	<LOQ	6,010	1,427	288,276	4,855
5	Day 4	Fortified Filler-1	1	10,317	9,356	7,345	10,482	11,123	18,259	11,352
5	Day 4	Fortified Filler-1	2	10,055	9,278	7,406	10,323	10,652	17,803	11,283
5	Day 4	Fortified Filler-1	3	10,302	9,196	6,875	10,182	10,532	17,879	11,573
6	Day 1	Filler-1	1	<LOQ	<LOQ	<LOQ	0,960	<LOQ	12,489	2,060
6	Day 1	Filler-1	2	<LOQ	<LOQ	<LOQ	0,986	<LOQ	13,241	2,048
6	Day 1	Filler-1	3	<LOQ	<LOQ	<LOQ	0,886	<LOQ	12,204	2,128
6	Day 1	Filler-2	1	<LOQ	<LOQ	1,146	2,982	1,727	516,142	9,986
6	Day 1	Filler-2	2	<LOQ	<LOQ	1,301	3,194	1,781	526,129	10,606
6	Day 1	Filler-2	3	<LOQ	<LOQ	1,146	3,136	1,728	525,089	9,007
6	Day 1	Filler-3	1	<LOQ	0,968	1,139	1,890	0,963	62,092	9,318
6	Day 1	Filler-3	2	<LOQ	0,904	1,094	1,881	1,009	61,917	9,834
6	Day 1	Filler-3	3	<LOQ	0,756	1,122	1,812	1,034	61,857	9,413
6	Day 1	Filler-4	1	<LOQ	<LOQ	<LOQ	5,310	1,048	418,315	4,423
6	Day 1	Filler-4	2	<LOQ	<LOQ	<LOQ	5,140	0,913	420,162	4,624
6	Day 1	Filler-4	3	<LOQ	<LOQ	<LOQ	5,326	1,170	423,057	4,696

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
6	Day 1	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
6	Day 1	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
6	Day 1	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
6	Day 2	Filler-1	1	<LOQ	<LOQ	<LOQ	0,974	<LOQ	12,203	2,053
6	Day 2	Filler-1	2	<LOQ	<LOQ	<LOQ	1,128	<LOQ	13,482	2,018
6	Day 2	Filler-1	3	<LOQ	<LOQ	<LOQ	1,085	<LOQ	13,307	1,973
6	Day 2	Filler-2	1	<LOQ	<LOQ	1,110	3,367	1,780	537,437	10,572
6	Day 2	Filler-2	2	<LOQ	<LOQ	1,272	3,402	1,648	535,516	11,080
6	Day 2	Filler-2	3	<LOQ	<LOQ	1,021	3,195	1,578	522,034	11,370
6	Day 2	Filler-3	1	<LOQ	0,887	1,264	1,911	0,764	63,411	10,697
6	Day 2	Filler-3	2	<LOQ	0,823	1,188	1,943	0,937	62,538	9,844
6	Day 2	Filler-3	3	<LOQ	<LOQ	1,299	2,069	0,887	65,885	10,127
6	Day 2	Filler-4	1	<LOQ	<LOQ	<LOQ	5,545	1,142	424,530	5,149
6	Day 2	Filler-4	2	<LOQ	<LOQ	<LOQ	5,241	1,146	402,186	5,273
6	Day 2	Filler-4	3	<LOQ	<LOQ	<LOQ	5,101	1,188	404,186	5,034
6	Day 2	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
6	Day 2	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
6	Day 2	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
6	Day 3	Filler-1	1	<LOQ	<LOQ	<LOQ	0,831	<LOQ	10,725	2,028
6	Day 3	Filler-1	2	<LOQ	<LOQ	<LOQ	0,949	<LOQ	11,983	2,014
6	Day 3	Filler-1	3	<LOQ	<LOQ	<LOQ	0,974	<LOQ	12,540	2,176
6	Day 3	Filler-2	1	<LOQ	<LOQ	1,102	3,388	1,593	519,628	10,689
6	Day 3	Filler-2	2	<LOQ	<LOQ	1,224	3,268	1,678	506,110	11,081
6	Day 3	Filler-2	3	<LOQ	<LOQ	1,053	3,226	1,664	499,808	10,746
6	Day 3	Filler-3	1	<LOQ	0,847	1,221	2,011	0,967	61,034	12,160
6	Day 3	Filler-3	2	<LOQ	0,769	1,372	2,019	0,877	60,464	11,487
6	Day 3	Filler-3	3	<LOQ	0,927	1,405	2,045	0,976	61,806	11,502
6	Day 3	Filler-4	1	<LOQ	<LOQ	<LOQ	5,729	0,768	414,719	5,099
6	Day 3	Filler-4	2	<LOQ	<LOQ	<LOQ	6,175	0,934	429,431	5,089
6	Day 3	Filler-4	3	<LOQ	<LOQ	<LOQ	5,742	0,978	417,182	5,065
6	Day 3	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
6	Day 3	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
6	Day 3	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
6	Day 4	Filler-1	1	<LOQ	<LOQ	<LOQ	0,948	<LOQ	10,941	2,688
6	Day 4	Filler-1	2	<LOQ	<LOQ	<LOQ	0,989	<LOQ	11,346	2,672
6	Day 4	Filler-1	3	<LOQ	<LOQ	<LOQ	0,984	<LOQ	11,308	2,788
6	Day 4	Filler-2	1	<LOQ	<LOQ	0,943	3,330	1,618	489,354	10,972
6	Day 4	Filler-2	2	<LOQ	<LOQ	0,978	3,231	1,663	497,450	11,302

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
6	Day 4	Filler-2	3	<LOQ	<LOQ	0,952	3,327	1,617	494,171	11,922
6	Day 4	Filler-3	1	<LOQ	<LOQ	1,194	1,985	0,756	58,600	12,201
6	Day 4	Filler-3	2	<LOQ	<LOQ	1,180	1,960	0,827	59,812	12,015
6	Day 4	Filler-3	3	<LOQ	<LOQ	1,148	1,967	0,855	57,400	12,161
6	Day 4	Filler-4	1	<LOQ	<LOQ	<LOQ	5,593	0,961	389,170	4,958
6	Day 4	Filler-4	2	<LOQ	<LOQ	<LOQ	5,711	0,998	405,422	4,926
6	Day 4	Filler-4	3	<LOQ	<LOQ	<LOQ	5,929	0,957	409,028	4,967
6	Day 4	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
6	Day 4	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
6	Day 4	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
7	Day 1	Filler-1	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12,749	2,194
7	Day 1	Filler-1	2	<LOQ	<LOQ	<LOQ	0,762	<LOQ	12,762	2,242
7	Day 1	Filler-1	3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11,100	1,948
7	Day 1	Filler-2	1	<LOQ	<LOQ	1,123	2,788	2,010	509,695	12,031
7	Day 1	Filler-2	2	<LOQ	<LOQ	1,115	2,569	2,168	509,872	11,628
7	Day 1	Filler-2	3	<LOQ	<LOQ	1,016	2,512	2,007	514,887	11,379
7	Day 1	Filler-3	1	<LOQ	<LOQ	1,365	1,420	1,115	59,527	9,474
7	Day 1	Filler-3	2	<LOQ	<LOQ	1,797	1,604	1,039	63,213	10,281
7	Day 1	Filler-3	3	<LOQ	<LOQ	1,412	1,477	0,974	60,096	9,950
7	Day 1	Filler-4	1	<LOQ	<LOQ	<LOQ	4,296	1,400	418,151	5,243

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
7	Day 1	Filler-4	2	<LOQ	<LOQ	<LOQ	4,926	1,318	434,497	5,704
7	Day 1	Filler-4	3	<LOQ	<LOQ	<LOQ	4,687	1,484	429,138	5,270
7	Day 1	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
7	Day 1	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
7	Day 1	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
7	Day 2	Filler-1	1	<LOQ	<LOQ	<LOQ	0,864	<LOQ	13,633	2,469
7	Day 2	Filler-1	2	<LOQ	<LOQ	<LOQ	0,989	<LOQ	16,789	3,061
7	Day 2	Filler-1	3	<LOQ	<LOQ	<LOQ	0,844	<LOQ	15,729	2,678
7	Day 2	Filler-2	1	<LOQ	<LOQ	0,829	2,580	2,153	489,530	12,080
7	Day 2	Filler-2	2	<LOQ	<LOQ	1,030	3,270	2,732	508,712	14,730
7	Day 2	Filler-2	3	<LOQ	<LOQ	1,200	3,311	2,564	539,386	14,708
7	Day 2	Filler-3	1	<LOQ	<LOQ	1,229	1,821	1,267	78,942	12,369
7	Day 2	Filler-3	2	<LOQ	<LOQ	1,479	2,049	1,465	90,479	14,045
7	Day 2	Filler-3	3	<LOQ	<LOQ	1,303	2,002	1,419	87,067	13,624
7	Day 2	Filler-4	1	<LOQ	<LOQ	<LOQ	4,773	1,415	468,464	5,907
7	Day 2	Filler-4	2	<LOQ	<LOQ	<LOQ	4,167	1,483	421,223	5,186
7	Day 2	Filler-4	3	<LOQ	<LOQ	<LOQ	4,342	1,484	387,553	5,193
7	Day 2	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
7	Day 2	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
7	Day 2	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
7	Day 3	Filler-1	1	<LOQ	<LOQ	<LOQ	0,769	<LOQ	12,775	2,239
7	Day 3	Filler-1	2	<LOQ	<LOQ	<LOQ	0,769	<LOQ	13,205	2,353
7	Day 3	Filler-1	3	<LOQ	<LOQ	<LOQ	0,800	<LOQ	12,985	2,379
7	Day 3	Filler-2	1	<LOQ	<LOQ	0,854	2,817	2,246	595,778	12,488
7	Day 3	Filler-2	2	<LOQ	<LOQ	1,006	2,478	2,242	590,102	11,569
7	Day 3	Filler-2	3	<LOQ	<LOQ	0,828	2,676	2,180	548,279	12,235
7	Day 3	Filler-3	1	<LOQ	<LOQ	1,047	1,565	1,147	67,012	10,639
7	Day 3	Filler-3	2	<LOQ	<LOQ	1,521	1,453	1,075	58,843	9,916
7	Day 3	Filler-3	3	<LOQ	<LOQ	1,669	1,591	1,259	67,617	11,817
7	Day 3	Filler-4	1	<LOQ	<LOQ	<LOQ	4,679	1,470	464,113	5,696
7	Day 3	Filler-4	2	<LOQ	<LOQ	<LOQ	4,465	1,514	455,928	5,711
7	Day 3	Filler-4	3	<LOQ	<LOQ	<LOQ	4,539	1,552	436,556	5,631
7	Day 3	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
7	Day 3	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
7	Day 3	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
7	Day 4	Filler-1	1	<LOQ	<LOQ	<LOQ	0,842	<LOQ	13,982	2,499
7	Day 4	Filler-1	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12,178	2,192
7	Day 4	Filler-1	3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	10,704	2,026
7	Day 4	Filler-2	1	<LOQ	<LOQ	0,864	2,322	2,255	492,924	11,309

Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
7	Day 4	Filler-2	2	<LOQ	<LOQ	0,798	2,302	1,968	533,437	10,379
7	Day 4	Filler-2	3	<LOQ	<LOQ	0,823	2,253	2,157	520,681	10,351
7	Day 4	Filler-3	1	<LOQ	<LOQ	1,232	1,459	1,027	59,937	10,108
7	Day 4	Filler-3	2	<LOQ	<LOQ	1,151	1,319	0,967	57,495	9,345
7	Day 4	Filler-3	3	<LOQ	<LOQ	1,523	1,590	1,068	67,457	11,196
7	Day 4	Filler-4	1	<LOQ	<LOQ	<LOQ	5,120	1,893	494,071	6,391
7	Day 4	Filler-4	2	<LOQ	<LOQ	<LOQ	4,562	1,470	468,382	5,885
7	Day 4	Filler-4	3	<LOQ	<LOQ	<LOQ	4,753	1,521	433,423	5,693
7	Day 4	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
7	Day 4	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
7	Day 4	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
8	Day 1	Filler-1	1	<LOQ	<LOQ	<LOQ	1,2640	<LOQ	11,362	2,040
8	Day 1	Filler-1	2	<LOQ	<LOQ	<LOQ	1,301	<LOQ	11,452	2,272
8	Day 1	Filler-1	3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12,116	2,140
8	Day 1	Filler-2	1	<LOQ	<LOQ	1,271	3,660	2,156	454,838	12,160
8	Day 1	Filler-2	2	<LOQ	<LOQ	<LOQ	3,699	1,958	434,947	11,429
8	Day 1	Filler-2	3	<LOQ	<LOQ	<LOQ	3,614	2,103	521,119	11,946
8	Day 1	Filler-3	1	<LOQ	<LOQ	1,275	2,227	1,260	51,663	10,330
8	Day 1	Filler-3	2	<LOQ	<LOQ	<LOQ	2,274	<LOQ	54,363	10,383
8	Day 1	Filler-3	3	<LOQ	<LOQ	<LOQ	2,196	<LOQ	51,559	10,705

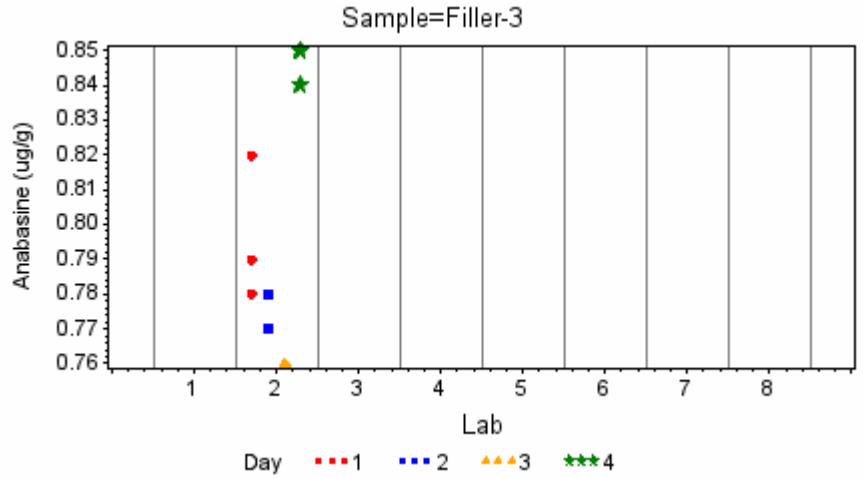
Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
8	Day 1	Filler-4	1	<LOQ	<LOQ	<LOQ	6,421	1,271	397,721	5,213
8	Day 1	Filler-4	2	<LOQ	<LOQ	<LOQ	6,742	1,419	413,895	5,323
8	Day 1	Filler-4	3	<LOQ	<LOQ	<LOQ	6,710	1,476	407,906	5,680
8	Day 1	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
8	Day 1	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
8	Day 1	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
8	Day 2	Filler-1	1	<LOQ	<LOQ	<LOQ	1,398	<LOQ	13,788	2,261
8	Day 2	Filler-1	2	<LOQ	<LOQ	<LOQ	1,305	<LOQ	12,204	2,329
8	Day 2	Filler-1	3	<LOQ	<LOQ	<LOQ	1,280	<LOQ	11,580	2,081
8	Day 2	Filler-2	1	<LOQ	<LOQ	<LOQ	3,517	2,263	516,411	12,270
8	Day 2	Filler-2	2	<LOQ	<LOQ	<LOQ	3,354	2,085	502,732	12,725
8	Day 2	Filler-2	3	<LOQ	<LOQ	<LOQ	3,985	2,554	499,002	11,993
8	Day 2	Filler-3	1	<LOQ	<LOQ	<LOQ	2,638	<LOQ	61,917	11,229
8	Day 2	Filler-3	2	<LOQ	<LOQ	1,308	2,827	1,570	63,459	12,253
8	Day 2	Filler-3	3	<LOQ	<LOQ	1,458	2,487	1,391	57,557	12,815
8	Day 2	Filler-4	1	<LOQ	<LOQ	<LOQ	7,953	1,311	444,819	6,279
8	Day 2	Filler-4	2	<LOQ	<LOQ	<LOQ	6,283	1,270	366,605	5,214
8	Day 2	Filler-4	3	<LOQ	<LOQ	<LOQ	7,834	1,310	443,354	6,587
8	Day 2	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA

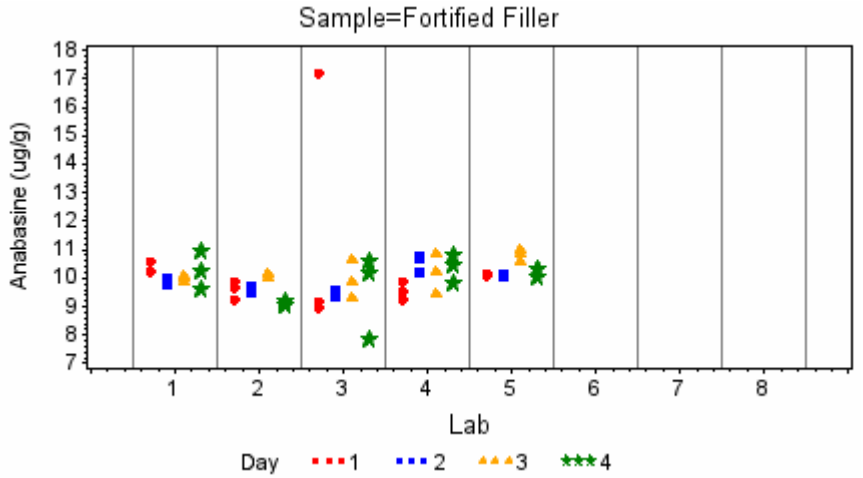
Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
8	Day 2	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
8	Day 2	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
8	Day 3	Filler-1	1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11,194	1,097
8	Day 3	Filler-1	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	12,265	1,481
8	Day 3	Filler-1	3	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	11,786	1,810
8	Day 3	Filler-2	1	<LOQ	<LOQ	<LOQ	3,362	<LOQ	527,654	13,307
8	Day 3	Filler-2	2	<LOQ	<LOQ	<LOQ	3,306	<LOQ	518,001	12,734
8	Day 3	Filler-2	3	<LOQ	<LOQ	<LOQ	2,393	<LOQ	392,274	9,339
8	Day 3	Filler-3	1	<LOQ	<LOQ	<LOQ	2,026	<LOQ	57,229	11,552
8	Day 3	Filler-3	2	<LOQ	<LOQ	<LOQ	1,989	<LOQ	60,435	12,712
8	Day 3	Filler-3	3	<LOQ	<LOQ	<LOQ	1,839	<LOQ	57,398	12,206
8	Day 3	Filler-4	1	<LOQ	<LOQ	<LOQ	4,974	<LOQ	322,416	3,814
8	Day 3	Filler-4	2	<LOQ	<LOQ	<LOQ	5,312	<LOQ	366,788	4,453
8	Day 3	Filler-4	3	<LOQ	<LOQ	<LOQ	5,607	<LOQ	350,147	3,884
8	Day 3	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
8	Day 3	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
8	Day 3	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA
8	Day 4	Filler-1	1	<LOQ	<LOQ	<LOQ	1,557	<LOQ	11,690	2,965
8	Day 4	Filler-1	2	<LOQ	<LOQ	<LOQ	1,5773	<LOQ	14,624	2,737

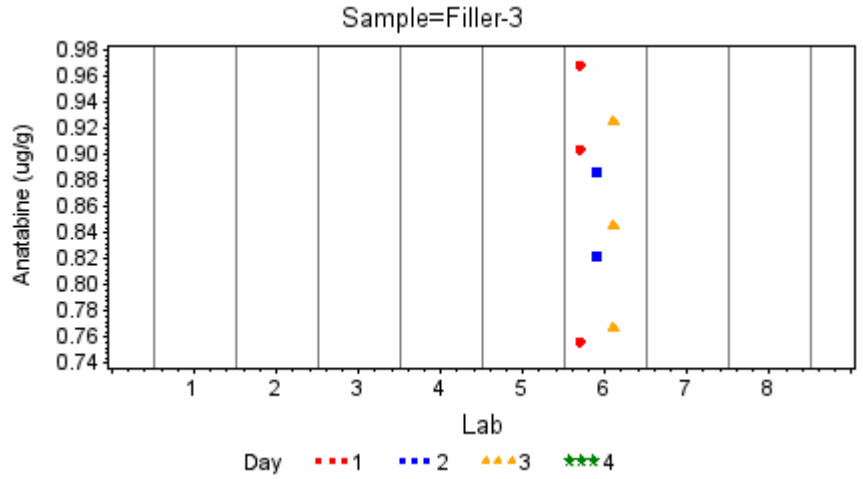
Lab #	Day	Sample	Rep	Anabasine (µg/g)	Anatabine (µg/g)	β-Nicotyrine (µg/g)	Cotinine (µg/g)	Myosmine (µg/g)	Nicotine-N'-Oxide (µg/g)	Nornicotine (µg/g)
8	Day 4	Filler-1	3	<LOQ	<LOQ	<LOQ	1,7314	<LOQ	15,328	2,891
8	Day 4	Filler-2	1	<LOQ	<LOQ	<LOQ	6,042	<LOQ	>500	13,615
8	Day 4	Filler-2	2	<LOQ	<LOQ	<LOQ	5,028	<LOQ	>500	13,489
8	Day 4	Filler-2	3	<LOQ	<LOQ	<LOQ	2,913	<LOQ	>500	12,479
8	Day 4	Filler-3	1	<LOQ	<LOQ	<LOQ	3,131	<LOQ	77,733	14,490
8	Day 4	Filler-3	2	<LOQ	<LOQ	<LOQ	5,597	<LOQ	73,077	17,000
8	Day 4	Filler-3	3	<LOQ	<LOQ	<LOQ	2,969	<LOQ	70,592	14,320
8	Day 4	Filler-4	1	<LOQ	<LOQ	<LOQ	5,201	<LOQ	298,610	3,330
8	Day 4	Filler-4	2	<LOQ	<LOQ	<LOQ	5,733	<LOQ	360,977	3,544
8	Day 4	Filler-4	3	<LOQ	<LOQ	<LOQ	6,537	<LOQ	380,307	4,549
8	Day 4	Fortified Filler-1	1	NA	NA	NA	NA	NA	NA	NA
8	Day 4	Fortified Filler-1	2	NA	NA	NA	NA	NA	NA	NA
8	Day 4	Fortified Filler-1	3	NA	NA	NA	NA	NA	NA	NA

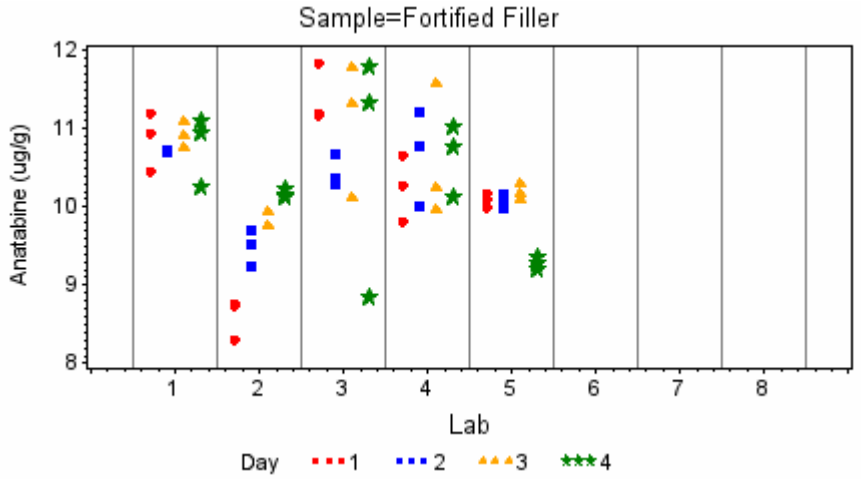
Note: <LOQ indicates values were found below the detection limit while NA indicates that the analytes were not detected.

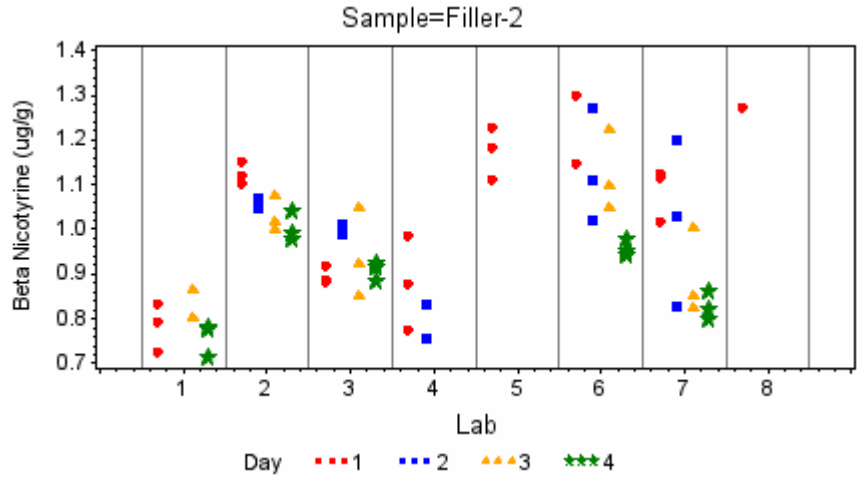
APPENDIX D: Raw Data Plots

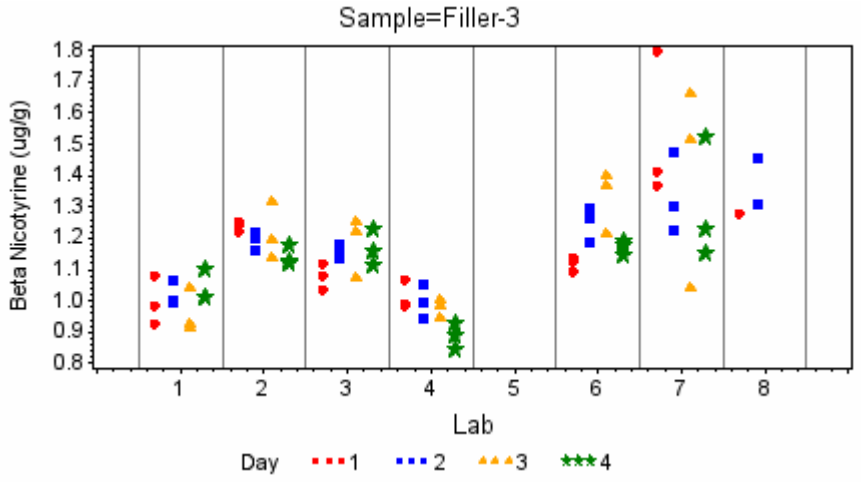


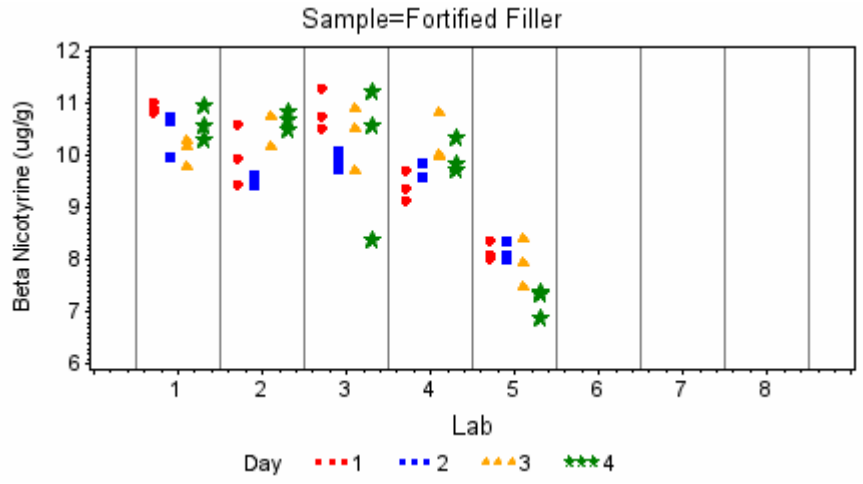


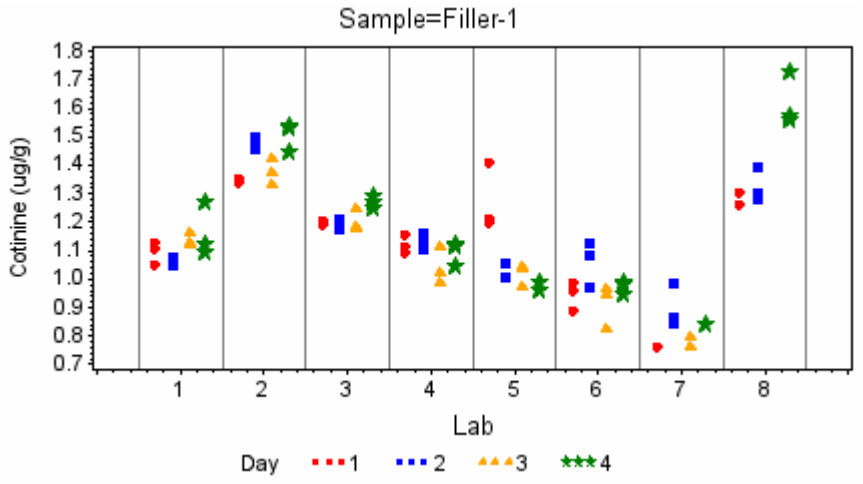


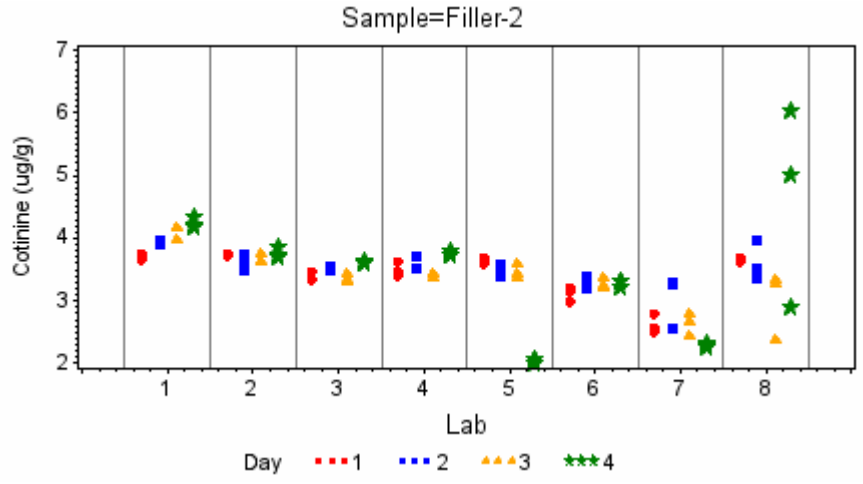


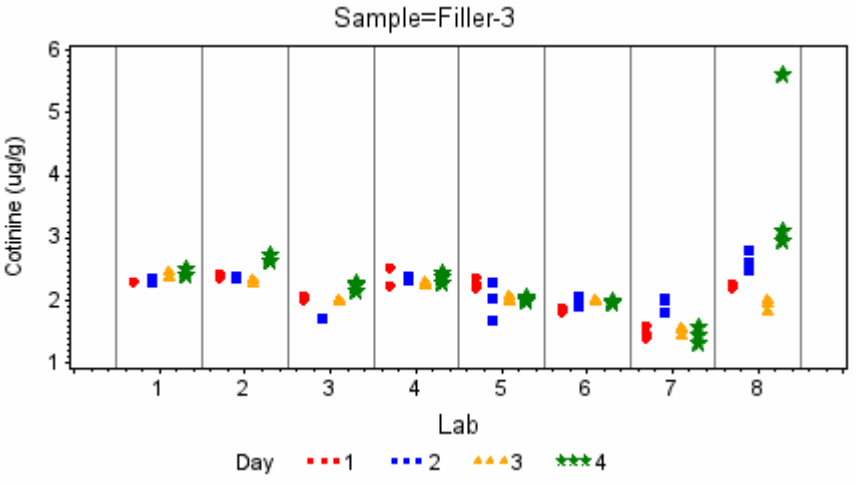

Sample=Filler-2
Beta Nicotyrine (ug/g)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
1 2 3 4 5 6 7 8
Lab
Day 1 2 3 4

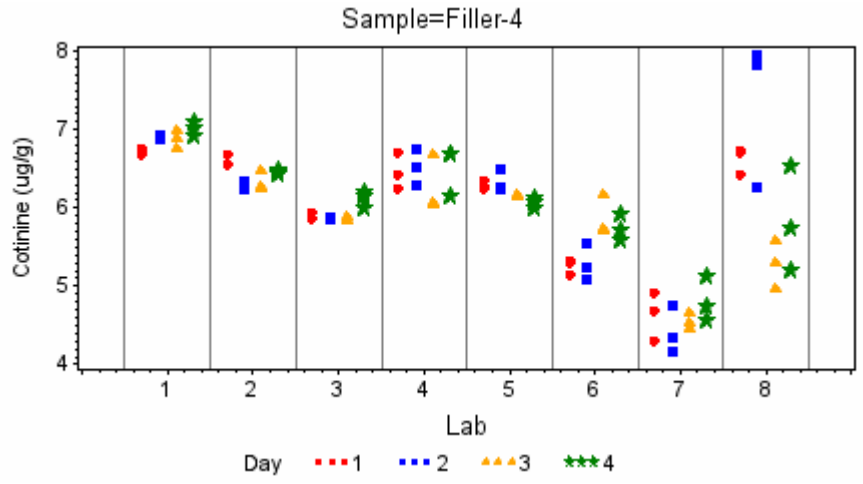

Sample=Filler-3
Beta Nicotyrine (ug/g)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
1 2 3 4 5 6 7 8
Lab
Day 1 2 3 4

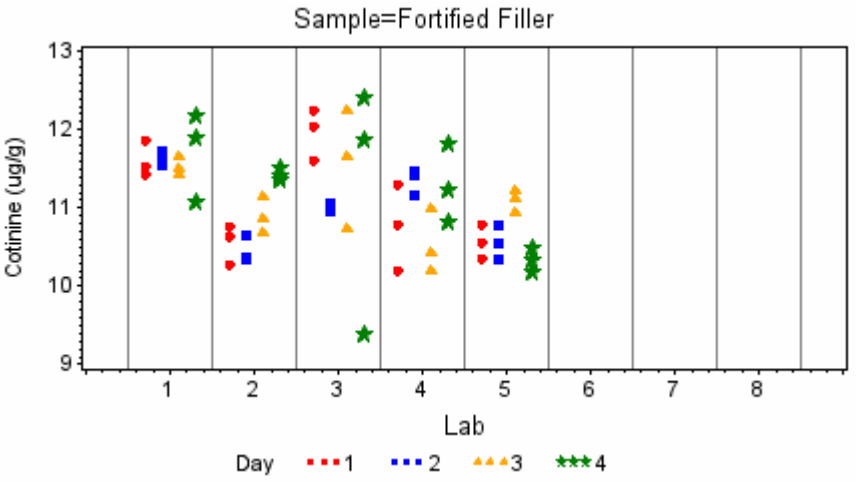

Sample=Fortified Filler
Beta Nicotyrine (ug/g)
12
11
10
9
8
7
6
1 2 3 4 5 6 7 8
Lab
Day 1 2 3 4

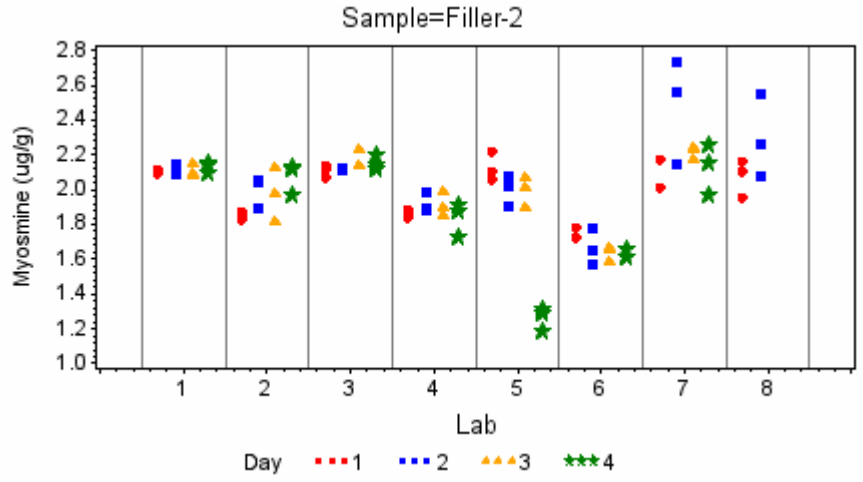

Sample=Filler-1
Cotinine (ug/g)
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
1 2 3 4 5 6 7 8
Lab
Day 1 2 3 4

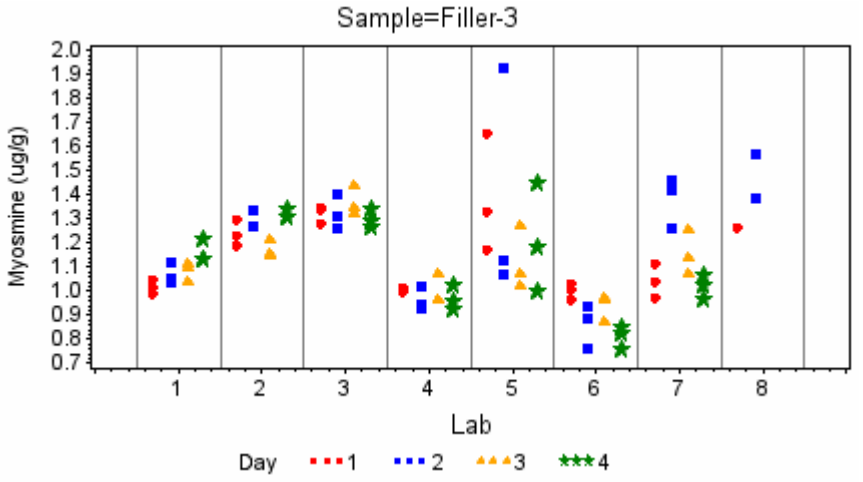

Sample=Filler-2
Cotinine (ug/g)
Lab
Day 1 2 3 4

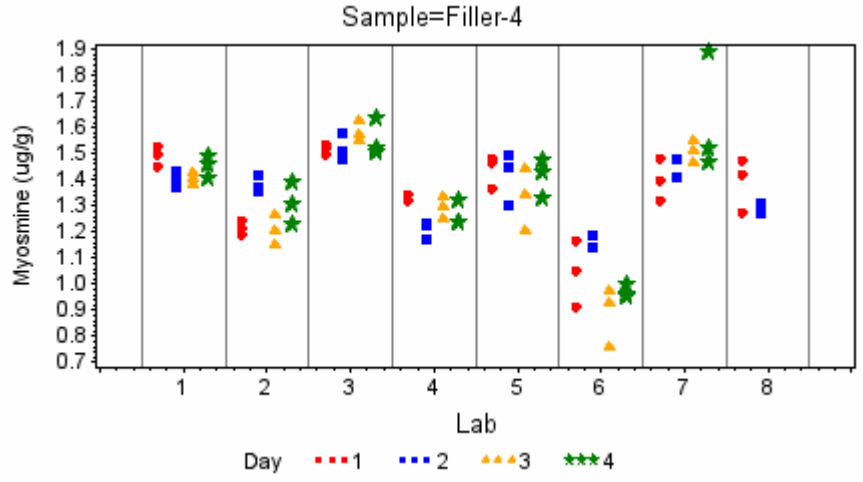

Sample=Filler-3
Cotinine (ug/g)
Lab
Day 1 2 3 4

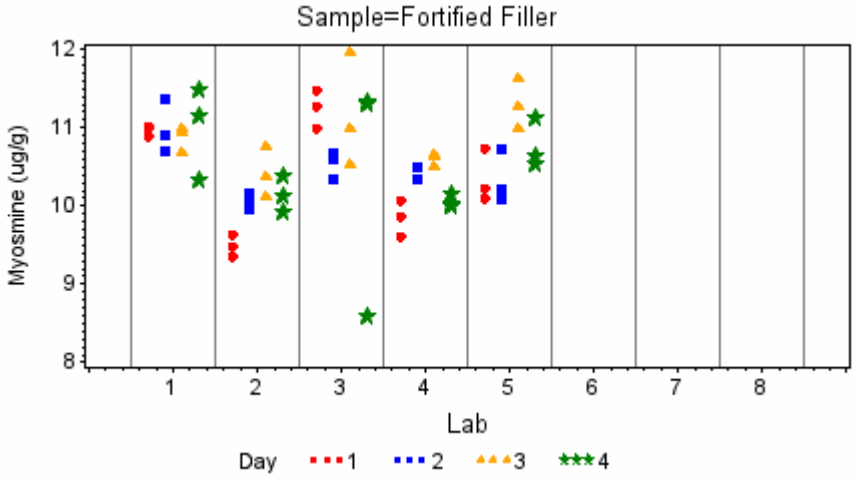

Sample=Filler-4
Cotinine (ug/g)
Lab
Day 1 2 3 4

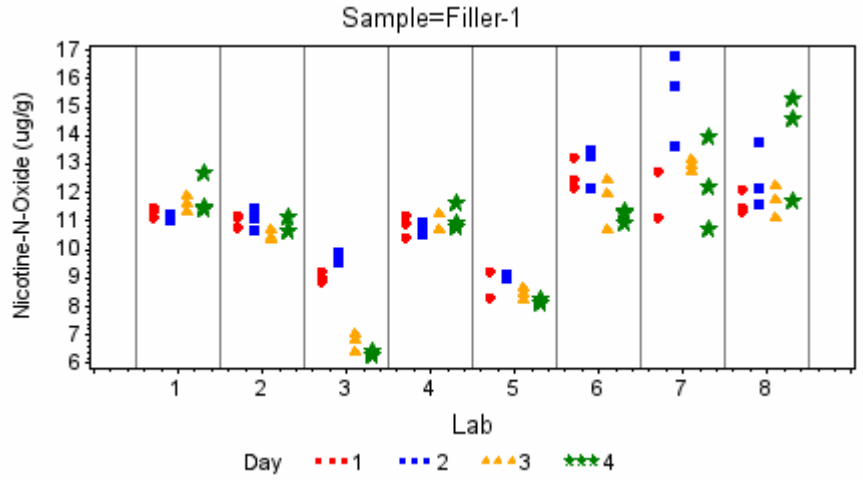

Sample=Fortified Filler
Cotinine (ug/g)
Lab
Day 1 2 3 4

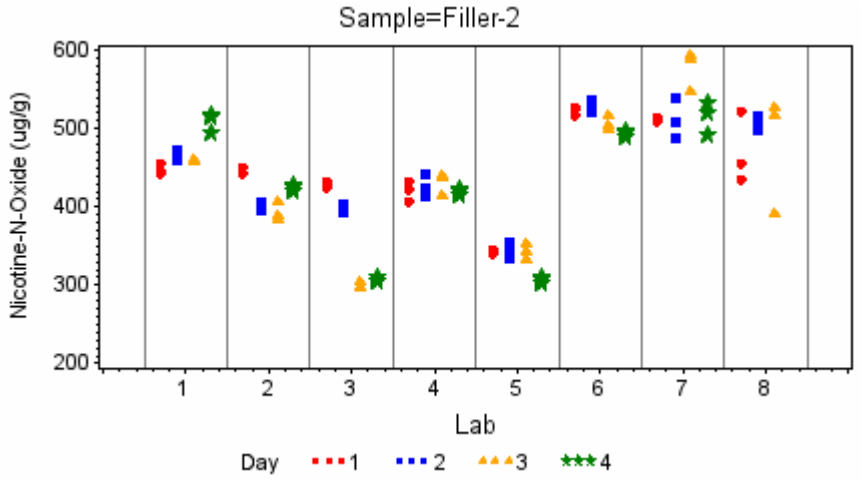

Sample=Filler-2
Myosmine (ug/g)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
1 2 3 4 5 6 7 8
Lab
Day 1 2 3 4

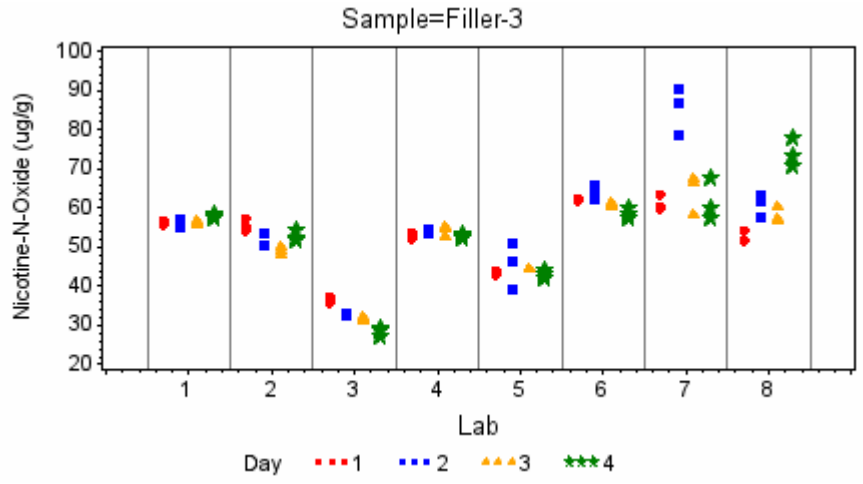

Sample=Filler-3
Myosmine (ug/g)
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
1 2 3 4 5 6 7 8
Lab
Day 1 2 3 4

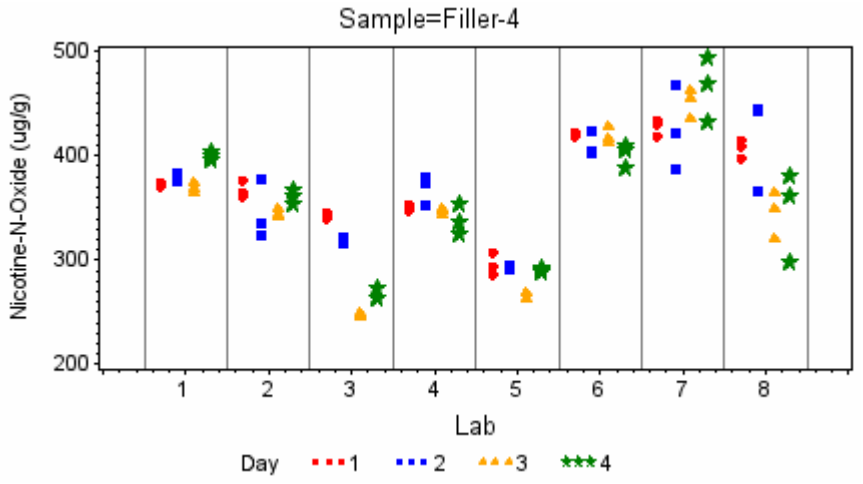

Sample=Filler-4
Myosmine (ug/g)
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
1 2 3 4 5 6 7 8
Lab
Day 1 2 3 4

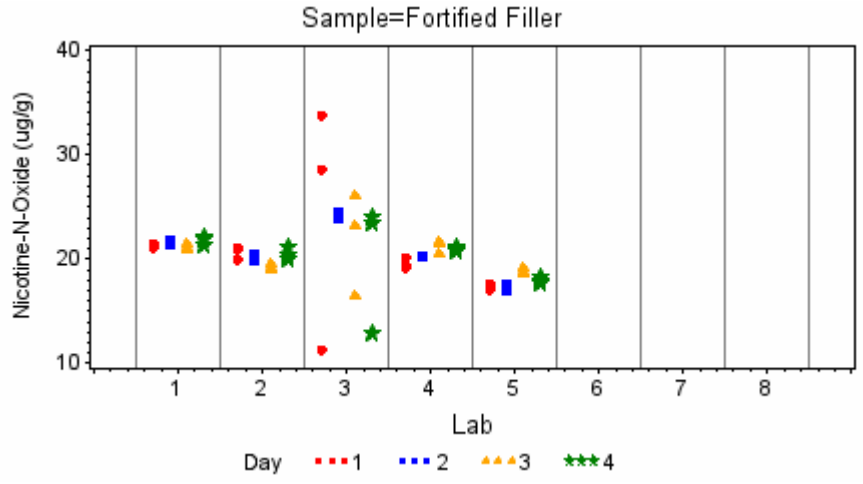

Sample=Fortified Filler
Myosmine (ug/g)
12
11
10
9
8
1 2 3 4 5 6 7 8
Lab
Day 1 2 3 4

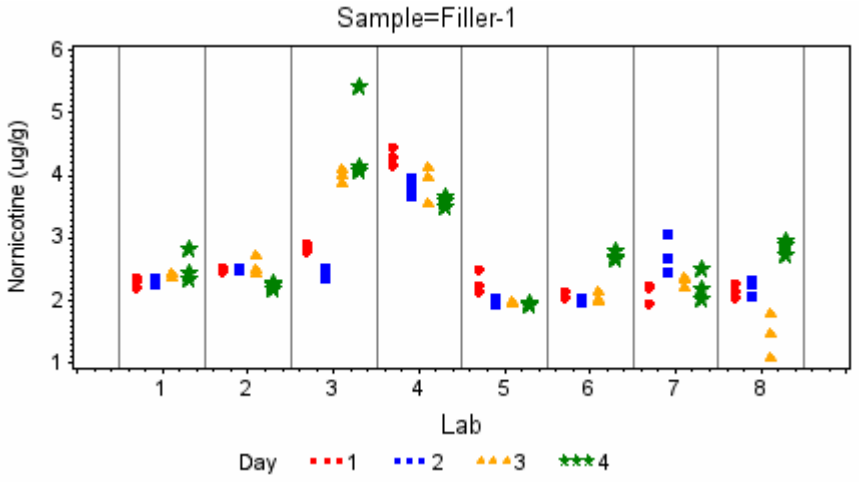

Sample=Filler-1
Nicotine-N-Oxide (ug/g)
17
16
15
14
13
12
11
10
9
8
7
6
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4

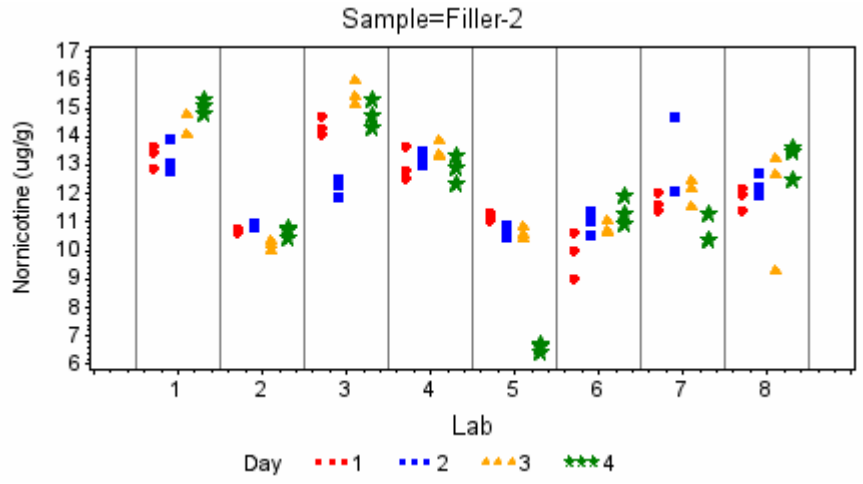

Sample=Filler-2
Nicotine-N-Oxide (ug/g)
600
500
400
300
200
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4

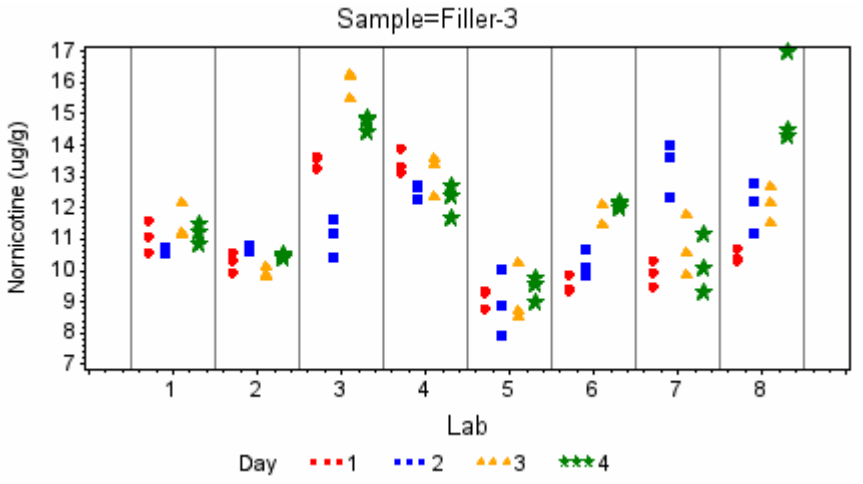

Sample=Filler-3
Nicotine-N-Oxide (ug/g)
100
90
80
70
60
50
40
30
20
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4

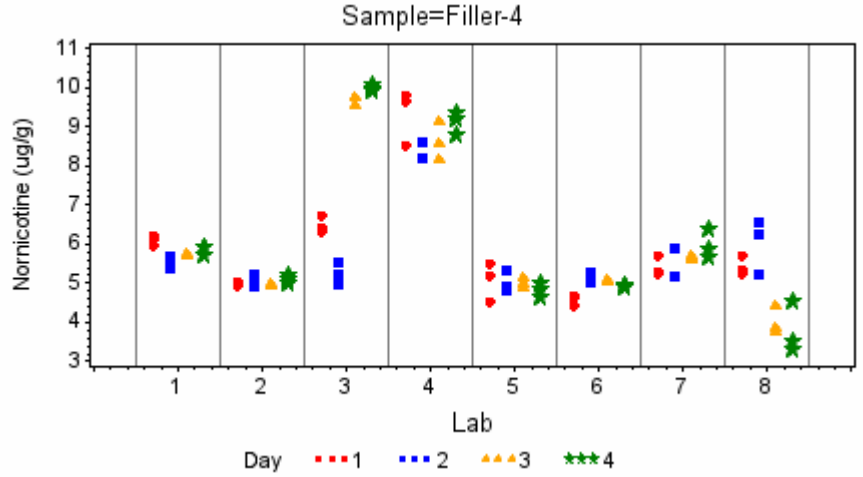

Sample=Filler-4
Nicotine-N-Oxide (ug/g)
500
400
300
200
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4

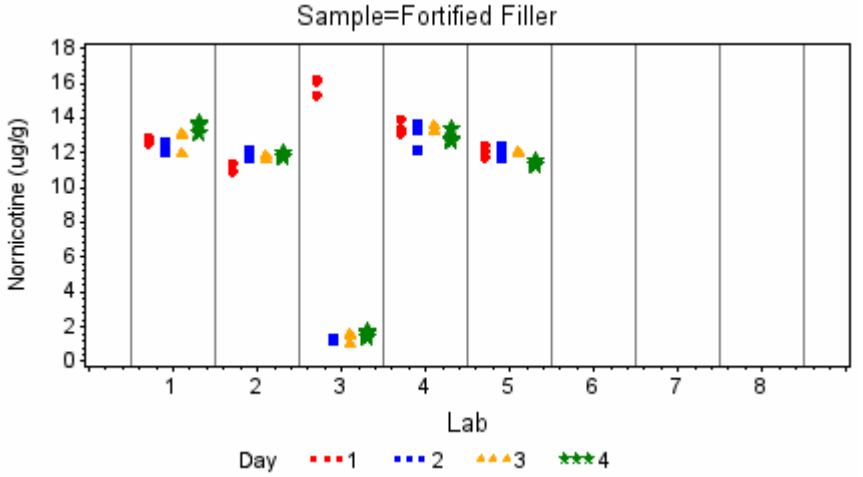

Sample=Fortified Filler
Nicotine-N-Oxide (ug/g)
40
30
20
10
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4


Sample=Filler-1
Nornicotine (ug/g)
6
5
4
3
2
1
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4

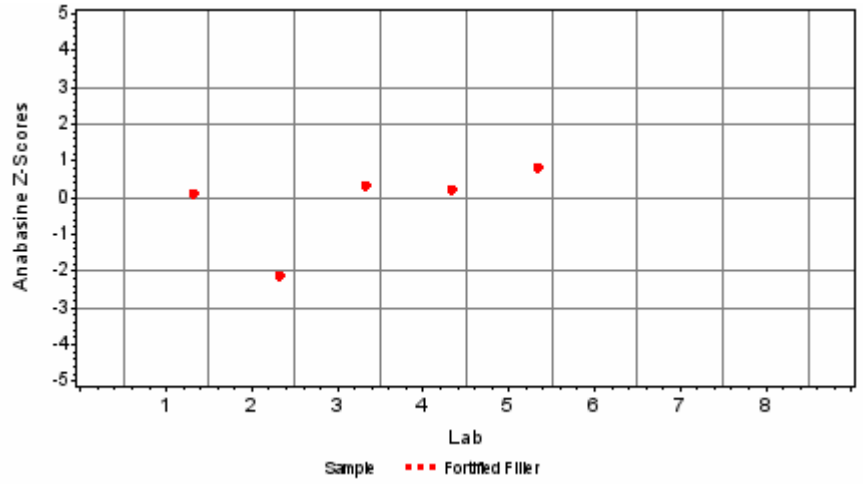

Sample=Filler-2
Nornicotine (ug/g)
17
16
15
14
13
12
11
10
9
8
7
6
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4

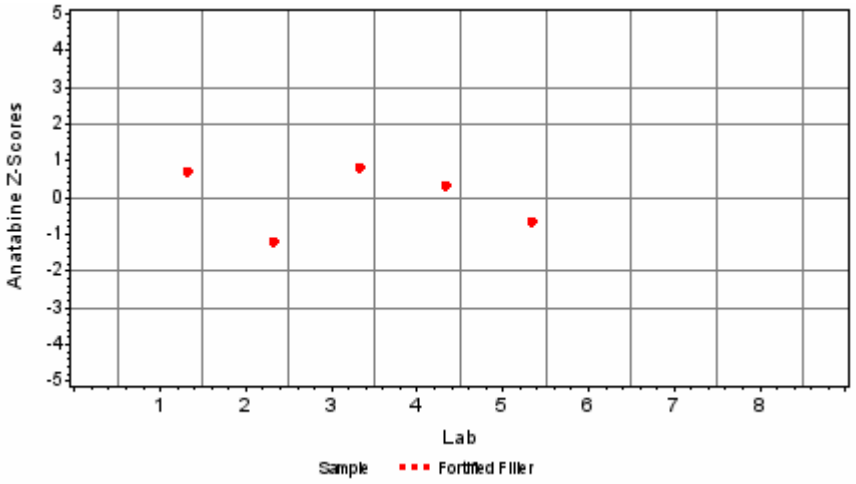

Sample=Filler-3
Nornicotine (ug/g)
17
16
15
14
13
12
11
10
9
8
7
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4

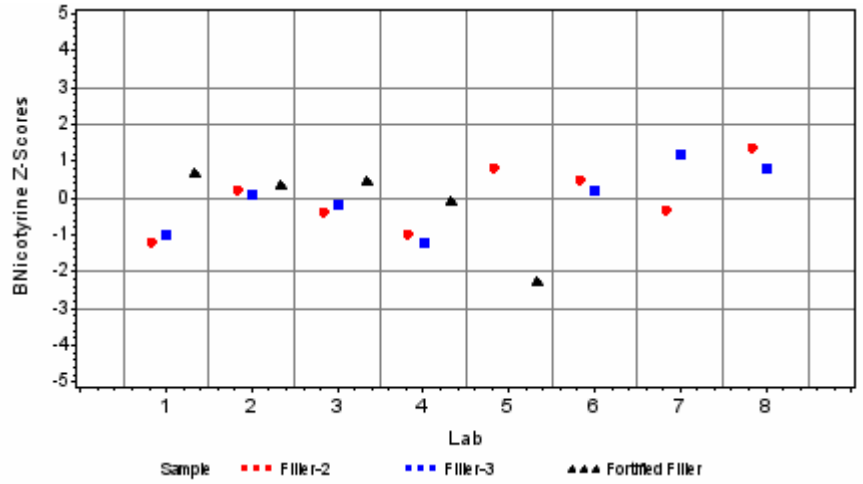

Sample=Filler-4
Nornicotine (ug/g)
11
10
9
8
7
6
5
4
3
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4

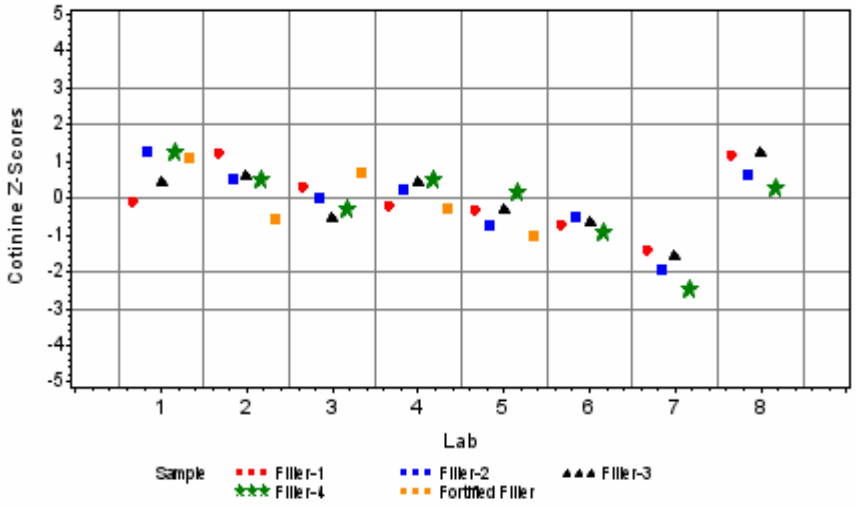

Sample=Fortified Filler
Nornicotine (ug/g)
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
Lab
Day
1
2
3
4

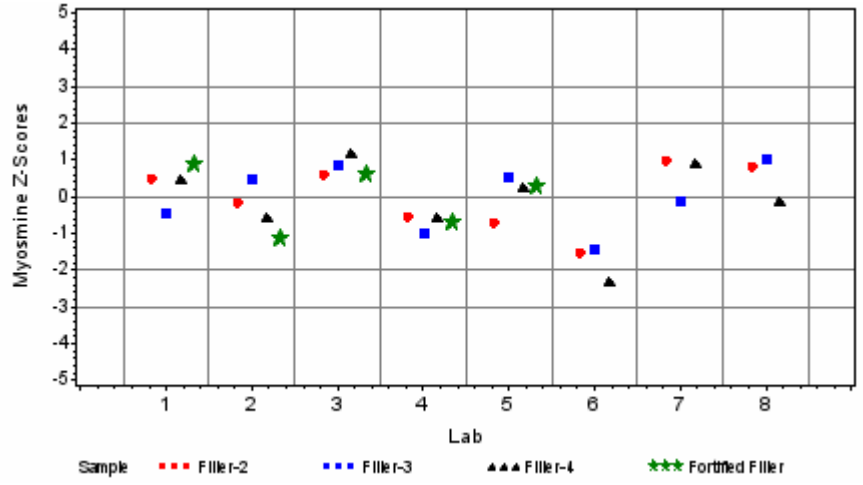
APPENDIX E: Z-Score Plots

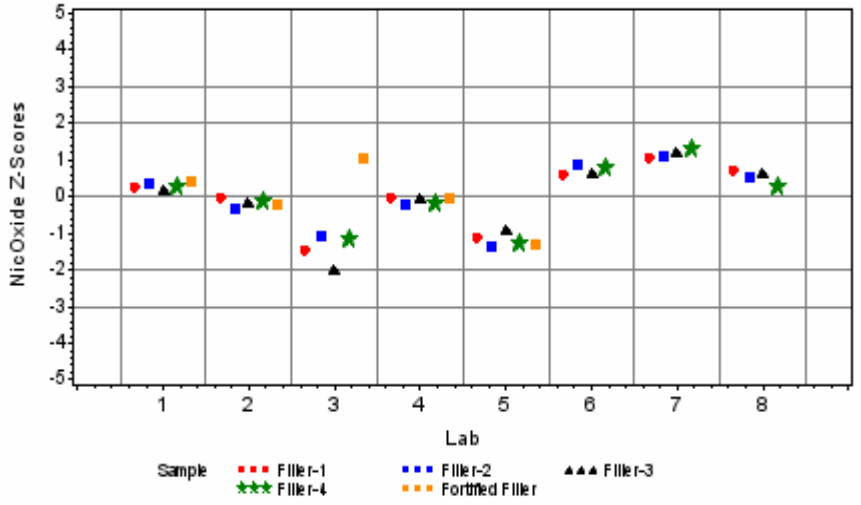


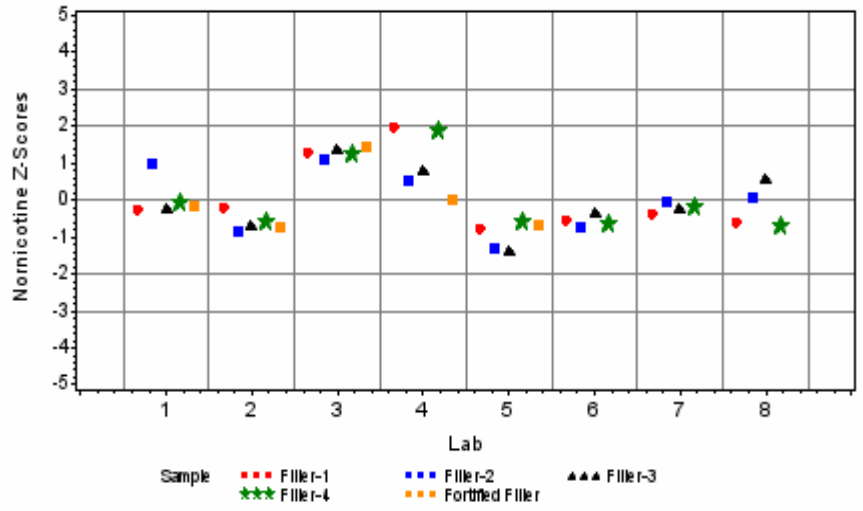







Myosmine Z-Scores
5
4
3
2
1
0
-1
-2
-3
-4
-5
1
2
3
4
5
6
7
8
Lab
Sample
Filler-2
Filler-3
Filler-4
Fortified Filler


NicOxide Z-Scores
5
4
3
2
1
0
-1
-2
-3
-4
-5
1
2
3
4
5
6
7
8
Lab
Sample
Filler-1
Filler-2
Filler-3
Filler-4
Fortified Filler


Nornicotine Z-Scores
5
4
3
2
1
0
-1
-2
-3
-4
-5
1
2
3
4
5
6
7
8
Lab
Sample
Filler-1
Filler-2
Filler-3
Filler-4
Fortified Filler