E-Vapour Sub-Group

Technical Report

2021 Collaborative Study for the Determination of Tobacco-Specific Nitrosamines in E-Liquids

November 2022

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1. Summary

At the spring 2021 virtual CORESTA E-Vapour Sub-Group (EVAP) meeting, the group approved the initiation of a collaborative study for the determination of tobacco-specific nitrosamines (TSNAs) in e-liquid products. The compounds include 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-nitrosonornicotine (NNN), *N*-nitrosoanabasine (NAB), and *N*-nitrosoanatabine (NAT) which were determined following a proposed recommended method. The results of the study demonstrate that the proposed recommended method is suitable for the determination of select TSNAs in e-liquid products.

2. Introduction

In January 2021, a survey of the analytical capabilities of the E-Vapour Sub-Group (EVAP) pertaining to the analysis of tobacco-specific nitrosamines was conducted. The results of this survey indicated a high amount of overlap between sample extraction and mode of analysis (analogous to CRM N° 72). Following approval from the E-Vapour Sub-Group (EVAP), a New Work Item Proposal (NWIP) was submitted to the Scientific Commission (SC) for the development of an analytical method suitable for the analysis of tobacco-specific nitrosamines in electronic cigarette liquids (e-liquids). The NWIP was approved by the SC in May 2021. During the spring EVAP meeting, a tentative timeline was discussed and following NWIP approval, laboratories stated their intention for participation in the study.

The participating laboratories agreed to determine the amounts of the analytes of interest in four e-liquids. As TSNAs are not expected to be present at significant amounts, each e-liquid was fortified at a different concentration with the target compounds (NAT, NAB, NNK, NNN). This was done to ensure that a measurable amount of each compound would be present for analysis.

The collaborative study was completed between January 2021 (survey sent out) and December 2021 (statistical analysis) with nine (9) laboratories participating. Draft results were presented at the EVAP meeting in February 2022. The purpose of this study was to evaluate the repeatability and reproducibility (r & R) values of the methodology and propose a new CORESTA Recommended Method (CRM) for the determination of tobacco-specific nitrosamines in e-liquids.

3. Organisation

3.1 Participants

Ten (10) laboratories agreed to participate in the study with nine (9) laboratories reporting results. A list of the participating laboratories is provided in Table 1. The laboratories are listed in alphabetical order. Numerical codes were assigned to each laboratory and do not correspond to the order shown in the table below.

Table 1: List of Participating Laboratories (listed in alphabetical order)

Participating Laboratories
Enthalpy Analytical, LLC
Imperial Brands Hamburg
ITG Brands
JT-SPAC
JTI-Oekolab
KT&G
RJ Reynolds Tobacco Company
Shenzhen Smoore Technology Limited
Zhengzhou Tobacco Research Institute (ZTRI) of CNTC

3.2 Protocol

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

3.2.1 Study Samples

Study e-liquids were provided by Alternative Ingredients, Inc. Laboratories were requested to store the samples at ambient conditions. The samples are identified in Table 2.

Table 2: Sample Identification

Sample ID	Flavour Profile	Target Nicotine (%w/w)	Target Propylene Glycol (%w/w)	Target Glycerol (%w/w)	Target Water (%w/w)	Target NAT, NNK, NNN/NAB Conc. (ng/g)
3128701 50:50 VG/PG	Unflavoured	0,0	50	50	0,0	-
3128701 CS-T 50:50 VG/PG	Unflavoured	0,0	50	50	0,0	3,2; 3,2; 3,2/0,8
3128702 ART. TOP FLAVOR	Tobacco	0,0	50	50	0,0	-
3128702 CS-T ART. TOP FLAVOR	Tobacco	0,0	50	50	0,0	12; 12; 12/3
3128704 ART. TOP MENTHOL FLAVOR	Tobacco menthol	0,0	50	50	0,0	-
3128704 CS-T ART. TOP MENTHOL FLAVOR	Tobacco menthol	0,0	50	50	0,0	48; 48; 48/12
3128703 ART. SWEET TOP FLAVOR	Fruit/Sweet	0,0	50	50	0,0	-
3128703 CS-T ART. SWEET TOP FLAVOR	Fruit/Sweet	0,0	50	50	0,0	80; 80; 80/20

Note: Samples were shipped without nicotine. Each participating lab was required to add nicotine prior to starting sample preparation.

3.2.2 Analysis

Each participating lab received two samples for each flavour profile, one that had been fortified with TSNAs, and one without. It was requested that all samples be fortified with nicotine to a concentration of approximately 1,5 % prior to sample extraction/analysis. Samples were to be stored at ambient temperature prior to analysis. Participants were asked to extract three replicates for all samples and to analyse samples according to the method given in Appendix B.

3.2.3 Deviations

Participating laboratories were requested to document any deviations from the protocol and the applicable draft CORESTA method and to submit any deviations with their results. Five laboratories reported minor deviations:

- **Lab 1:** The instrument LOQ used for NAT, NNK and NNN was higher than the LOQ listed in the provided method. The concentration of analyte in sample 3128701 CS-T 50:50 VG/PG was below the LOQ for these analytes. Data was still used for statistical analysis. Acetic acid was used as part of mobile phase A instead of ammonium acetate and mobile phase gradient used matched CRM N° 75 instead of provided method.
- **Lab 2:** Reported using a different mobile phase and a higher concentration of internal standard in samples. The reported LOQ was also lower than what is listed in the provided draft method for NAT, NNK, and NNN (~0,02 ng/mL vs 0,048 ng/mL). Results consistent with other labs.
- **Lab 4:** Highest concentration standard was 4 ng/mL and 1 ng/mL for NNN/NNK/NAT and NAB, respectively instead of 12 ng/mL and 3 ng/mL. Results were consistent with other participating labs.
- **Lab 7:** Reported using a different HPLC gradient.
- **Lab 8:** Was not able to obtain nicotine to fortify samples, results still included in statistical analysis.

4. Study Data

The raw data set is provided in Appendix C. Each analysis includes three replicates.

5. Statistical Analysis

The statistical analysis was conducted in basic conformance with ISO 5725-5:1998. This analysis procedure does not involve outlier detection, but, instead, uses calculation procedures that are resistant to the effects of outliers. The calculated results for repeatability (r) and reproducibility (R) are given below in section 5.1. Raw data plots that include all replicates are shown in Appendix D.

5.1 Calculation of Repeatability and Reproducibility

Table 3: Repeatability (r) and Reproducibility (R) Limits for NAT (ng/g)

Sample	Nominal Conc. (ng/g)	N° of Labs	Average (ng/g)	Repeatability		Reproducibility	
				r	r %	R	R %
3128701-Unflavoured	3,2	9	3,44	0,49	14,2	1,31	38,1
3128702-Tobacco	12	9	12,06	1,13	9,4	3,8	26,4
3128703-Sweet	80	9	77,94	4,03	5,2	18,77	24,1
3128704-Menthol	48	9	51,19	3,38	6,6	6,70	13,1

Table 4: Repeatability (r) and Reproducibility (R) Limits for NAB (ng/g)

Sample	Nominal Conc. (ng/g)	N° of Labs	Average (ng/g)	Repeatability		Reproducibility	
				r	r %	R	R %
3128701-Unflavoured	0,8	9	0,82	0,14	16,5	0,50	60,4
3128702-Tobacco	3,0	9	2,85	0,48	16,8	1,48	52,0
3128703-Sweet	20	9	19,86	2,28	11,5	7,46	37,6
3128704-Menthol	12	9	12,56	0,72	5,7	3,69	29,3

Table 5: Repeatability (r) and Reproducibility (R) Limits for NNK (ng/g)

Sample	Nominal Conc. (ng/g)	N° of Labs	Average (ng/g)	Repeatability		Reproducibility	
				r	r %	R	R %
3128701-Unflavoured	3,2	9	3,21	0,26	8,2	1,46	45,5
3128702-Tobacco	12	9	11,84	1,26	10,7	2,60	22,0
3128703-Sweet	80	9	80,55	3,61	4,5	15,79	19,6
3128704-Menthol	48	9	49,90	3,33	6,7	9,79	19,6

Table 6: Repeatability (r) and Reproducibility (R) Limits for NNN (ng/g)

Sample	Nominal Conc.(ng/g)	N° of Labs	Average (ng/g)	Repeatability		Reproducibility	
				r	r %	R	R %
3128701-Unflavoured	3,2	9	3,19	0,60	18,9	1,81	56,7
3128702-Tobacco	12	9	11,08	1,11	10,0	2,84	25,7
3128703-Sweet	80	9	78,25	5,22	6,7	18,15	23,2
3128704-Menthol	48	9	47,88	3,81	8,0	11,44	23,9

6. Data Interpretation

In this study, NAT, NNK, NNN, and NAB were added to each flavour profile at the concentrations listed in Table 2. For each flavour profile, a set of blank samples was also shipped to be extracted in triplicate. Each laboratory was asked to fortify the received samples with nicotine to a concentration of approximately 1,5 %. As the blank test samples are expected to have low levels of TSNAs, if any at all, any background is therefore likely to come from the added nicotine (depending on the source) or the internal standard used (lot purity). To compensate for this, blank subtraction was applied to the fortified samples, but only if the average background levels measured in the blank extracts were above the listed LOQ for each participating laboratory. Generally, most labs reported the blank data as below LOD (non-detect) or below LOQ. Three participants reported background levels of TSNAs above the reported LOQ and were therefore used for blank subtraction for their respective samples only. All data was reported on a per gram basis, correcting for any variation in sampling amongst the participating laboratories.

The summary of the r & R data can be found in Tables 3 through 6. The reproducibility values for this study ranged from 13,1 % to 60.4 % across all spiking levels and are comparable to those seen in CRM N° 72 for all analytes. Using the Horwitz-Thompson equation, which predicts the reproducibility standard deviation as a function of the concentration[1], the predicted reproducibility standard deviation would be 22 % for any analyte at a concentration below 120 ng/g or, equivalently, %R is predicted to be 2,8·22 % = 62 %.

The highest %R observed in this study (60,4 %) was for NAB at the lowest spiking level (0.8 ng/g) in product 3128701-Unflavoured. As the spiking concentration increased, the %R improved, as would be expected. By this standard, as well as the comparison to other CRMs, the method is deemed fit for purpose.

7. Recommendations

The study results were reviewed during the February 2022 EVAP Sub-Group meeting. It was decided that this collaborative study supported the publication of a CORESTA Recommended Method for the analysis of TSNAs in e-liquids.

[1] See "Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing", Michael Thompson, 2000, *Analyst*, 125, pp 385-386.

APPENDIX A: Study Protocol

CORESTA E-VAPOUR SUB-GROUP

Project Number: 304

Project Title: TSNA Method for E-Liquids

Type of Document: Protocol

Revision Date: September 28, 2021

Written by: Joseph Jablonski (Enthalpy Analytical, Study Coordinator) and I. Gene Gillman (Juul Labs, SG Coordinator)

1. Introduction

This protocol describes the study to for the analysis of tobacco-specific nitrosamines in e-liquid products. The analytes of interest include the following:

• 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK)
• *N*-Nitrosonornicotine (NNN)
• *N*-Nitrosoanabasine (NAB)
• *N*-Nitrosoanatabine (NAT)

2. Objective

The participating laboratories are to determine the amounts of the analytes of interest in the provided e-liquids to be examined. The participating laboratories will acquire the samples for this study from Alternative Ingredients (see below for information). Samples will be fortified prior to shipping. A draft method will be provided.

3. Time Schedule

Table 1. Projected Time Schedule and Data Reporting Table

Date	Activity
16 Jun 21-18 Jun 21	Distribute draft protocol and method
30 Jun 21	Laboratories state their intention to participate and order supplies
09 Jul 21	Distribute final protocol, method, and data reporting sheet
August 2021	Participants order and receive the samples
August-September 2021	Laboratories conduct the study
14 October 21	Laboratories submit results by this date
October 2021	Statistical evaluation and preparation of results
November/December 2021	Discuss results at 2021 Fall EVAP meeting

4. Participating Laboratories

Following receipt of this protocol, the participating laboratories in this study will confirm and notify Joe Jablonski and Gene Gillman of their active participation.

5. Samples

5.1 Selection

Each participating laboratory should request the following e-liquid products from Eduardo Berea from Alternative Ingredients Inc. Each sample will be fortified at a single level of NAB, NAT, NNK, and NNN and sent to participating laboratories. Unfortified samples will also be sent for analysis. In total, participating laboratories will receive eight (8) samples.

Table 2. 2021 CORESTA TSNA CS Products

Sample ID	Flavour Profile	Target Nicotine (%w/w)	Target Propylene Glycol (%w/w)	Target Glycerol (%w/w)	Target Water (%w/w)	Target NAT, NNK, NNN/NAB Conc. (ng/g)
3128701 50:50 VG/PG	Unflavoured	0,0	50	50	0,0	-
3128701 CS-T 50:50 VG/PG	Unflavoured	0,0	50	50	0,0	3,2/0,8
3128702 Art. Top Flavor	Tobacco	0,0	50	50	0,0	-
3128702 CS-T Art. Top Flavor	Tobacco	0,0	50	50	0,0	12/3
3128704 Art. Top Menthol Flavor	Tobacco menthol	0,0	50	50	0,0	-
3128704 CS-T Art. Top Menthol Flavor	Tobacco menthol	0,0	50	50	0,0	48/12
3128703 Art. Sweet Top Flavor	Fruit/Sweet	0,0	50	50	0,0	-
3128703 CS-T Art. Sweet Top Flavor	Fruit/Sweet	0,0	50	50	0,0	80/20

Note: Samples will be shipped without nicotine. Each participating lab will need to add nicotine prior to starting sample preparation (see section 5.4).

5.2 Supplies and Shipping

E-Liquids

Shipments of the 2021 TSNA CS products may be obtained through Eduardo Berea from Alternative Ingredients Inc.

Each participating laboratory will send its shipping address, person to whom delivery should be made, shipping account (FedEx International, DHS, UPS) arrangements, and any special delivery information to Eduardo Berea at the following email address:

Please use the following table:

Sample Required	EVAP TSNAs Collaborative Study
Company Name	
Address	
City	
State & ZIP	
Country	
Attention	
Phone	
Fax	
E-mail	
Shipping Account Information	
Special Delivery Information	

Laboratories will be notified 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 not submit data if they question the integrity of the samples they received.

5.3 Receipt

Upon receipt, the samples are to be stored at ambient temperature prior to testing. Received samples should remain unopened until testing.

5.4 Within Laboratory Sample Preparation

Each participating laboratory will be required to fortify the test samples with nicotine prior to starting sample preparation. Nicotine may be purchased from any supplier provided its purity is ≥ 99 %.

Each laboratory will receive two samples for each flavour profile, one which has been fortified with TSNAs, and one without. All samples will need to have nicotine added to them prior to analysis. The target nicotine concentration is 1,5 % and can be achieved by mixing 60 mg of nicotine with 3940 mg of the e-liquid sample. Mix thoroughly prior to use in the study. Once mixed, samples can be stored at ambient temperatures until analysis.

6. Analysis

For this study, each sample received will be prepared in triplicate. Three aliquots of 0,2 g shall be taken from each of the eight (8) received samples following nicotine addition. The samples will be analysed by the provided method. Samples where the values of analyte are greater than ten times the expected value are to be considered outliers and the analysis repeated.

7. Data Reporting

Enter the final results and any comments into the provided data reporting spreadsheet (2021_EVAP_TSNAs_ELiquid_Data_Reporting_Sheet). The final reporting spreadsheet and any questions should be forwarded to the study coordinator Joseph Jablonski.

- Data shall be reported to four significant figures
- The data reporting sheet requires the LOD and LOQ be reported for each analyte.
- If data are below a quantitation limit, report the estimated analytical result for the specific analyte and make a note beside the cell that the result is below the quantitation limit.
- If data are below a detection limit, report the result for the specific analyte as below the detection limit (<LOD).
- Report any relevant deviations

8. Statistical Analysis

A statistical analysis in general conformance with ISO 5725-2:1994 and ISO/TR 22971:2005 will be conducted.

9. Tabulation and Presentation of the Data

The data will be coded by laboratory number rather than laboratory identity. The code will be provided to the respective participating laboratory along with the tabulated data. Draft results will be presented at the fall 2021 EVAP meeting.

APPENDIX B: Analysis Method

Draft

METHOD FOR THE ANALYSIS
OF TOBACCO-SPECIFIC NITROSAMINES IN E-LIQUIDS

0. INTRODUCTION

In 2021, the CORESTA E-Vapour Sub-Group conducted a collaborative study for the determination of tobacco-specific nitrosamines (TSNAs) in e-liquids using liquid chromatography with mass spectrometry. The TSNAs examined as part of this study included *N*-nitrosonornicotine (NNN), 4-(*N*-methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), *N*-nitrosoanatabine (NAT), and *N*-nitrosoanabasine (NAB). Ten laboratories participated in the study. The method used for this study was shown to be appropriate for the analysis of TSNAs in e-liquids.

1. SCOPE

The purpose of this document is to describe the procedures used for the determination of tobacco-specific nitrosamines (TSNAs) in electronic cigarette liquids (e-liquids). The TSNAs determined with this method are: N-nitrosonornicotine (NNN), 4-(N-methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT), and N-nitrosoanabasine (NAB). The e-liquids used may be of any flavour, including extracted tobacco-derived flavours.

2. NORMATIVE REFERENCES

2.1 CORESTA Guide N° 18 Technical Guide for Sample Handling and Sample Collection of E-Cigarettes and E-Vapour Generating Products

2.2 CORESTA Recommended Method N° 72 Determination of Tobacco-Specific Nitrosamines in Tobacco and Tobacco Products by LC-MSMS

2.3 CORESTA Recommended Method N° 75 Determination of Tobacco-Specific Nitrosamines in Mainstream Cigarette Smoke by LC-MSMS

3. SUMMARY OF METHOD

Following the addition of deuterium-labelled internal standards, TSNAs are extracted from the e-liquid into an aqueous buffer solution and filtered. Samples are analysed by Liquid Chromatography – Triple Quadrupole Mass Spectrometry (LC-MS/MS). The results are reported in ng/g of e-liquid.

4. SAFETY

All laboratory personnel performing this method should be familiar with appropriate laboratory practices and health and safety measures. Prior to the handling of any of the chemicals described within this document, personnel should familiarize themselves with the appropriate safety data sheets (SDS). Appropriate personal protective equipment (PPE) should be worn at all times and include, but are not limited to, laboratory coat, safety glasses/goggles, and gloves.

The TSNAs discussed in this method (NNN, NNK, NAT, and NAB) are reported to be carcinogens or suspected carcinogens. As such, appropriate safety measures, including appropriate PPE, must be taken when handling neat materials or any solution which contains the TSNAs analysed in this method.

5. EQUIPMENT AND SUPPLIES

High performance liquid chromatograph coupled to tandem mass spectrometer (LC-MS/MS) with an electrospray ionization source capable of performing the method described herein.

5.1 C18 HPLC column, 2,5 µm particle size, 2,1 mm x 50 mm, or equivalent[1].

5.2 Analytical balance capable of 0,1 mg accuracy.

5.3 Orbital shaker, wrist action shaker, or similar.

5.4 Vortex.

5.5 Class A glassware (volumetric flasks, volumetric pipettes, graduated cylinders).

5.6 Gas-tight syringes.

5.7 Amber vials with screw caps, 8 mL, or similar.

5.8 Adjustable pipettes.

5.9 Glass autosampler vials with caps.

5.10 0,45 µm Nylon filter or equivalent.

5.11 Disposable syringes.

6. REAGENTS

Unless specified, all reagents should be recognized as analytical grade or better where available. Solvents should be HPLC-grade or better. Note: ISO 17034 certified solutions may be used in place of neat standard materials.

6.1 N-Nitrosonornicotine (NNN); CAS # 16543-55-8; (min. 98 %)

6.2 N-Nitrosoanatabine (NAT); CAS # 71267-22-6; (min. 98 %)

6.3 N-Nitrosoanabasine (NAB); CAS # 1133-64-8; (min. 98 %)

6.4 4-(N-Methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK); CAS # 64091-91-4 (min. 98 %)

6.5 N-Nitrosonornicotine-2,4,5,6-d4 (NNN-d4); CAS # 66148-19-4; (min. 98 %)

6.6 N-Nitrosoanatabine-2,4,5,6-d4 (NAT-d4); CAS # 1020719-69-0; (min. 98 %)

6.7 N-Nitrosoanabasine-2,4,5,6-d4 (NAB-d4); CAS # 1020719-68-9; (min. 98 %)

6.8 4-(N-Methylnitrosoamino)-1-(3-pyridyl)-1-butanone-2,4,5,6-d4 (NNK-d4); CAS # 764661-24-7; (min. 98 %)

6.9 Ammonium acetate (min 98 %) CAS # 631-61-8

6.10 Acetic acid (min 98 %) CAS # 64-19-7

6.11 Acetonitrile (HPLC-grade) CAS # 75-05-8

6.12 Methanol (HPLC-grade) CAS # 67-56-1

6.13 Deionized water (≥ 18,20 MΩ·cm)

[1] Waters XBridge BEH C18® is an example of a commercially available column which is suitable for use. Note that this example does not constitute an endorsement of this product, but is merely given as an example. Other columns may be used with this method, provided they have been demonstrated to be fit for analysis and that analytes and internal standards are sufficiently resolved from interferences.

7. PREPARATION OF SOLUTIONS

7.1 Extraction Solution (100 mM ammonium acetate solution).

7.2 Weigh 15,4 g ± 0,05 g of ammonium acetate and transfer into a 2000 mL volumetric flask. Dilute to mark with deionized water and mix well.

7.3 Mobile Phase A (10 mM ammonium acetate in water).

7.4 Weigh 0,7708 g ± 0,02 g of ammonium acetate and transfer into a 1000 mL volumetric flask. Dilute to mark with deionized water and mix well.

7.5 Mobile Phase B (0,1% acetic acid solution in methanol).

7.6 Add 1 mL of acetic acid into a 1000 mL volumetric flask. Dilute to mark with methanol and mix well.

7.7 7.430/70 v/v Acetonitrile/Deionized Water.

7.8 Combine 150 mL acetonitrile with 350 mL of deionized water in a 500 mL glass screw cap bottle.

7.9 Mix well prior to use.

7.10 7.510/90 v/v Acetonitrile/10 mM ammonium acetate.

7.11 Combine 100 mL acetonitrile with 900 mL of 10 mM ammonium acetate solution (prepared in section 7.2) in a 1000 mL glass screw cap bottle.

7.12 Mix well prior to use.

8. PREPARATION OF STANDARDS

8.1 Preparation of Internal Standard solutions

8.1.1 Primary Solution

Weigh approximately 10 mg each of NNN-d4, NAT-d4, NAB-d4, and NNK-d4 into individual 10 mL volumetric flasks.

Fill to volume with acetonitrile and mix well.

The concentration will be approximately 1000 µg/mL.

8.1.2 Combined Secondary Internal Standard Solution

Transfer 2 mL of each primary solution of NNN-d4, NAT-d4, NAB-d4 and NNK-d4 into a 100 mL volumetric flask.

Dilute to volume with acetonitrile and mix well.

8.1.3 Internal Standard Spiking Solution

Transfer 2 mL of each primary solution of NNN-d4, NAT-d4, NAB-d4 and NNK-d4 into a 250 mL volumetric flask.

Dilute to volume with acetonitrile and mix well.

8.2 Preparation of Calibration Standard Solutions

8.2.1 Primary Stock Solutions

Weigh approximately 10 mg each of NNN, NAT, NAB, and NNK into individual 10 mL volumetric flasks and record the weight to 0,1 mg.

Fill to volume with acetonitrile and mix well.

The concentration should be approximately 1000 µg/mL.

8.2.2 Mixed Primary Stock Solution

Transfer 4 mL of each primary solution of NNN, NAT, NNK, and 1 mL of NAB into a 100 mL volumetric flask.

Dilute to volume with acetonitrile and mix well.

The concentration is approximately 40 µg/mL for NNN, NAT, NNK and 10 µg/mL for NAB.

8.2.3 Mixed Secondary Stock Solution

Transfer 2,5 mL of the mixed primary stock solution into a 250 mL volumetric flask.

Dilute to volume with 30/70 acetonitrile/deionized water and mix well.

The concentration is approximately 400 ng/mL for NNN, NAT, NNK, and 100 ng/mL for NAB.

8.2.4 Mixed Tertiary Stock Solution

Transfer 10 mL of the mixed secondary stock solution into a 100 mL volumetric flask.

Dilute to volume with 30/70 acetonitrile/deionized water and mix well.

The concentration is approximately 40 ng/mL for NNN, NAT, NNK, and 10 ng/mL for NAB.

8.3 TSNAs Calibration Standards

Prepare seven (7) calibration standards that cover the range of interest. An example of calibration standard preparation can be found in Table 1. The TSNA standards are prepared in separate flasks each containing a small amount of 10/90 acetonitrile/10 mM ammonium acetate solution (prepared in section 7.5) and the internal spiking solution amount listed in Table 1. Transfer the appropriate volume of the mixed tertiary stock solution given in Table 1 and fill to volume with 10/90 acetonitrile/10 mM ammonium acetate solution.

Table 1. Calibration Standard Preparation

Calibration Standard	Flask (mL)	3°Cal (mL) [see 8.2.4]	ISSS (mL) [see 8.1]	Calculated NAT, NNK, NNN Conc. (ng/mL)	Calculated NAB Conc. (ng/mL)
1	25	0,03	0,25	0,048	0,012
2	25	0,06	0,25	0,096	0,024
3	10	0,10	0,10	0,400	0,100
4	10	0,20	0,10	0,800	0,200
5	10	0,40	0,10	1,60	0,400
6	10	0,80	0,10	3,20	1,20
7	25	7,50	0,25	12,0	3,00

Note: Stock solutions of the individual TSNAs or internal standards in acetonitrile may be purchased at required levels.

Note: Linearity and range should be determined for every lab/instrument used for the quantitation of samples and should be appropriate for the types of samples to be analysed.

8.4 Storage

All standards and stocks prepared in this procedure are stable for up to six months when stored at or below 5° C.

9. PROCEDURES

9.1 Samples are to be stored and conditioned according to CORESTA Guide No. 18, Technical Guide for Sample Handling and Sample Collection of E-Cigarettes and E-Vapour Generating Products.

9.2 E-liquid extraction procedures are as follows (according to CORESTA Guide No. 18):

9.2.1 Remove all packaging and applicable endcaps and discard. Remove the mouth piece with a pair of needle nose pliers.

9.2.2 Place tweezers all the way to the bottom of the cartridge and twist the cartridge several times to free the substrate from the cartridge.

9.2.3 Unwrap the substrate and place in the barrel of a disposable syringe. Re-insert the plunger and press down on the syringe plunger to force the liquid into a non-hygroscopic, inert, sealable container appropriate for storage time and storage conditions.

9.2.4 Storage and handling of the e-liquids is dependent upon the intended analysis. However, many e-liquid compositions contain relatively volatile flavours and/or hygroscopic ingredients such as glycerol. Thus, samples should be stored or processed as quickly as possible in a manner that avoids prolonged exposure to the atmosphere.

Note: Some tanks/cartomizer may be sealed or tamper resistant. Liquid may be removed by centrifugation. Plastic tanks may be cut open using a small "PVC" pipe cutter.

9.3 Sample Extraction

9.3.1 Using an analytical balance, accurately transfer 200 µL of sample to a tared extraction vessel and record the weight to the nearest 0,1 mg.

9.3.2 Add 50 µL of the internal standard spiking solution using a calibrated pipette or equivalent.

9.3.3 Add 5 mL of extraction solution to each vessel and cap.

9.3.4 Vortex samples until mixed (at least 5 seconds).

9.3.5 Aliquot samples directly into an amber autosampler vial and cap.

9.3.5.1 If filtration is required, 0.45 µm Nylon syringe filters may be used and samples filtered directly into amber vials.

9.3.6 Sample extract is ready for analysis.

Samples that exceed calibration range for any TSNA compound require either of the following:

9.3.7a Sample preparation may be repeated using a greater volume of extraction solution, provided the appropriate amount of internal standard is used to give a concentration of 1,6 ng/mL.

9.3.7b Extracted samples may be diluted with extraction solution containing internal standard at a concentration of 1,6 ng/mL. Mix samples and proceed with reinjection.

10. DETERMINATION

Set up and operate the LC-MS/MS in accordance with the manufacturer's instructions.

10.1 Suggested Parameters

The following parameters are recommended for the LC system and may be modified to achieve better performance.

- Column temperature: 55,0 °C
- Injection volume: 10 µL
- Flow rate: 0,35 mL/min
- Mobile Phase A: 10 mM Ammonium Acetate in Water
- Mobile Phase B: 0,1 % (v/v) acetic acid in methanol

The following HPLC column gradient is suggested, but it may be modified as needed to achieve better performance

Table 2. HPLC Gradient

Time (min.)	Mobile Phase A (%)	Mobile Phase B (%)	Curve	Flow Rate (mL/min.)
Initial	97	3	Initial	0,35
1,00	97	3	Linear	0,35
4,00	10	90	Linear	0,35
4,01	1	99	Linear	0,35
5,75	1	99	Linear	0,35
5,90	97	3	Linear	0,35
9,00	97	3	Linear	0,35

10.2 MS/MS Parameters and Transitions

For this method, a triple quadrupole mass spectrometer shall be used and operated in positive electrospray mode using multiple reaction monitoring (MRM). The triple quadrupole should be carefully optimized for each analyte prior to use. Recommended precursor and product ions can be found in Table 3. Cone voltages and collision energies should be optimized on each instrument prior to analysis.

Table 3. Suggested Mass Transitions for TSNAs.

Analyte	Precursor Ion	Product Ion	Suggested Internal Standard
NAB	192	162	NAB-d4
NAB-d4	196	166	-
NAT	190	160	NAT-d4
NAT-d4	194	164	-
NNK	208	122	NNK-d4
NNK-d4	212	126	-
NNN	178	148	NNN-d4
NNN-d4	182	152	-

10.3 System Suitability

Prior to injection of samples, system performance must be evaluated. This evaluation should include assessing sensitivity, chromatographic performance, and any other criterion to ensure optimal performance of the LC-MS/MS system.

10.4 Calibration Curve

The initial calibration curve must consist of at least five calibration standards for each analyte. The recommended curve type is linear with the origin excluded. A weighting of 1/x may be applied. The calibration curve must have a coefficient of determination (r^2) of 0,995 or greater. For the calibration curve to be considered acceptable, each standard must be within 15 % of their target value.

10.5 Calculations

The concentration of each TSNA is present in the sample extract is determined by using the internal standard calibration curve. The value is converted to sample concentration as follows:

$$\text{E-Liquid Conc. (ng/g)} = \frac{\text{Conc.(ng/mL)} \times \text{Extraction Volume (mL)}}{\text{Sample Weight (g)}}$$

10.6 Quality Control

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

Appendix A

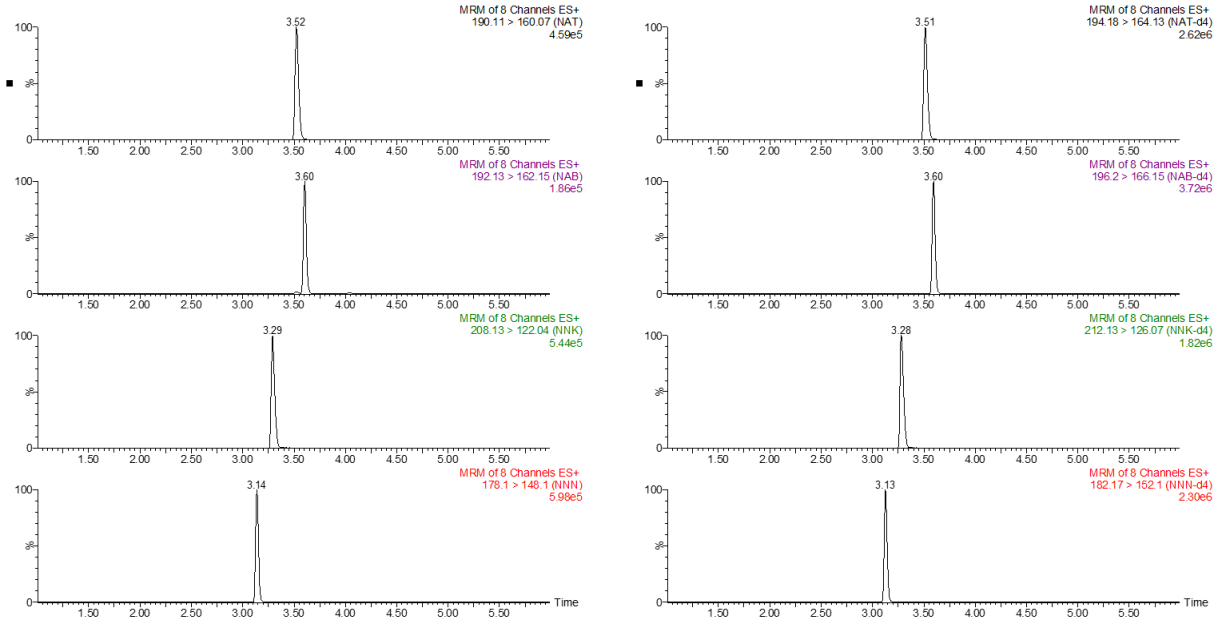


Figure 1 – Example of a MRM-chromatogram for a TSNA standard

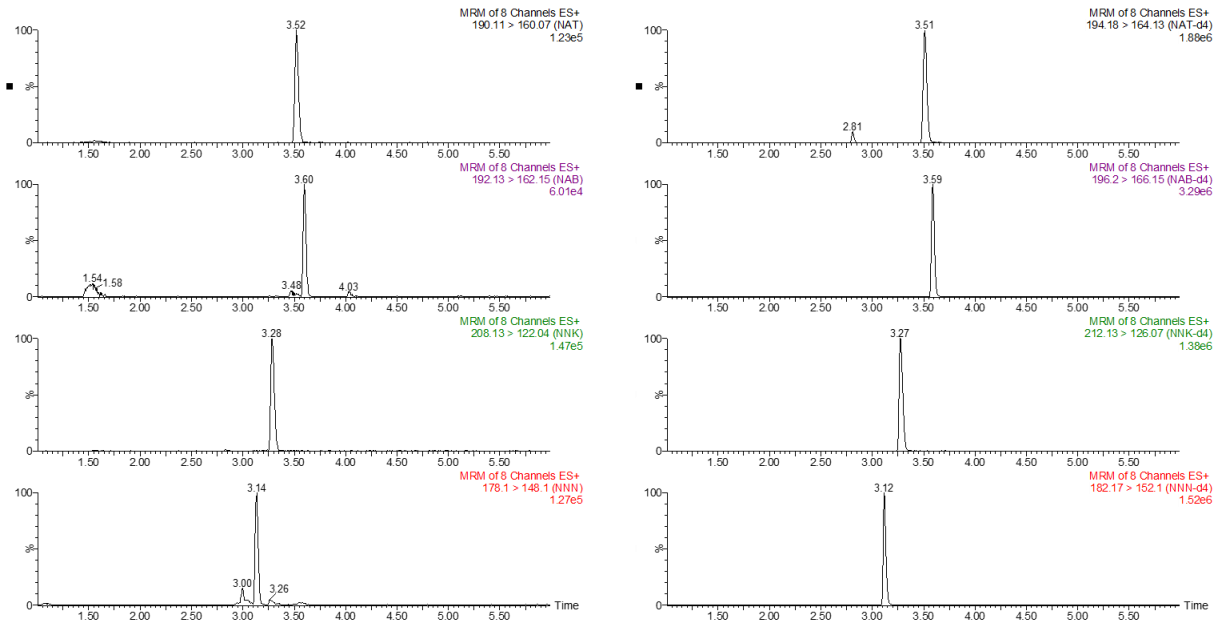


Figure 2 – Example of a MRM-chromatogram for an e-liquid fortified with NAT, NAB, NNK and NNN

APPENDIX C: Raw Data

Sample	Flavour	No Spiking					
		Lab Code	Nominal Spike level (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)	NNN (ng/g)
3128701	Unflavoured	1	0	0,000†	<LOD	0,000†	0,000†
3128701	Unflavoured	1	0	0,000†	<LOD	0,000†	0,000†
3128701	Unflavoured	1	0	0,000†	<LOD	0,000†	0,000†
3128701	Unflavoured	2	0	<LOD	<LOD	<LOD	<LOD
3128701	Unflavoured	2	0	<LOD	<LOD	<LOD	<LOD
3128701	Unflavoured	2	0	<LOD	<LOD	<LOD	<LOD
3128701	Unflavoured	3	0	0,134*	0,019*	0,104*	<LOD
3128701	Unflavoured	3	0	0,126*	0,025*	0,090*	<LOD
3128701	Unflavoured	3	0	0,124*	0,020*	0,093*	<LOD
3128701	Unflavoured	4	0	<LOD	<LOD	<LOD	0,297*
3128701	Unflavoured	4	0	<LOD	<LOD	<LOD	0,330*
3128701	Unflavoured	4	0	<LOD	<LOD	<LOD	0,289*
3128701	Unflavoured	5	0	<LOD	<LOD	<LOD	1,384
3128701	Unflavoured	5	0	<LOD	<LOD	<LOD	1,454
3128701	Unflavoured	5	0	<LOD	<LOD	<LOD	1,427
3128701	Unflavoured	6	0	<LOD	3,353	<LOD	12,174
3128701	Unflavoured	6	0	0,957*	3,259	<LOD	11,395
3128701	Unflavoured	6	0	0,807*	3,206	<LOD	11,278
3128701	Unflavoured	7	0	1,224	0,000†	1,145	0,000†
3128701	Unflavoured	7	0	1,428	0,000†	1,313	0,000†
3128701	Unflavoured	7	0	1,417	0,000†	1,417	0,000†
3128701	Unflavoured	8	0	2,338	1,113	0,480	2,553
3128701	Unflavoured	8	0	2,408	1,078	0,465	2,198
3128701	Unflavoured	8	0	2,284	1,096	0,377	2,155
3128701	Unflavoured	9	0	<LOD	<LOD	<LOD	<LOD
3128701	Unflavoured	9	0	<LOD	<LOD	<LOD	<LOD
3128701	Unflavoured	9	0	<LOD	<LOD	<LOD	<LOD
3128702	Tobacco	1	0	0,000†	<LOD	0,000†	0,000†
3128702	Tobacco	1	0	0,000†	<LOD	0,000†	0,000†
3128702	Tobacco	1	0	0,000†	<LOD	0,000†	0,000†
3128702	Tobacco	2	0	<LOD	<LOD	<LOD	<LOD
3128702	Tobacco	2	0	<LOD	<LOD	<LOD	<LOD
3128702	Tobacco	2	0	<LOD	<LOD	<LOD	<LOD
3128702	Tobacco	3	0	0,125*	0,018*	0,112*	<LOD
3128702	Tobacco	3	0	0,104*	<LOD	0,096*	<LOD
3128702	Tobacco	3	0	0,118*	0,024*	0,104*	<LOD
3128702	Tobacco	4	0	<LOD	<LOD	<LOD	0,297*
3128702	Tobacco	4	0	<LOD	<LOD	<LOD	0,278*
3128702	Tobacco	4	0	<LOD	<LOD	<LOD	0,317*
3128702	Tobacco	5	0	<LOD	<LOD	<LOD	1,246

Sample	Flavour	No Spiking					
		Lab Code	Nominal Spike level (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)	NNN (ng/g)
3128702	Tobacco	5	0	<LOD	<LOD	<LOD	1,301
3128702	Tobacco	5	0	<LOD	<LOD	<LOD	1,287
3128702	Tobacco	6	0	0,727*	2,909	<LOD	9,620
3128702	Tobacco	6	0	0,607*	3,081	0,817*	11,181
3128702	Tobacco	6	0	1,019*	3,381	0,764*	11,904
3128702	Tobacco	7	0	1,047	0,000†	1,068	0,000†
3128702	Tobacco	7	0	1,239	0,000†	1,215	0,000†
3128702	Tobacco	7	0	1,133	0,000†	1,112	0,000†
3128702	Tobacco	8	0	2,207	1,023	0,219	2,437
3128702	Tobacco	8	0	2,560	0,848	0,662	2,554
3128702	Tobacco	8	0	2,381	0,995	0,216	2,072
3128702	Tobacco	9	0	<LOD	<LOD	<LOD	<LOD
3128702	Tobacco	9	0	<LOD	<LOD	<LOD	<LOD
3128702	Tobacco	9	0	<LOD	<LOD	<LOD	<LOD
3128703	Sweet	1	0	0,000†	<LOD	0,000†	0,000†
3128703	Sweet	1	0	0,000†	<LOD	0,000†	0,000†
3128703	Sweet	1	0	0,000†	<LOD	0,000†	0,000†
3128703	Sweet	2	0	<LOD	<LOD	<LOD	<LOD
3128703	Sweet	2	0	<LOD	<LOD	<LOD	<LOD
3128703	Sweet	2	0	<LOD	<LOD	<LOD	<LOD
3128703	Sweet	3	0	0,117*	0, <LOD	0,101*	<LOD
3128703	Sweet	3	0	0,125*	0,021*	0,110*	<LOD
3128703	Sweet	3	0	0,112*	0,021*	0,105*	<LOD
3128703	Sweet	4	0	<LOD	<LOD	<LOD	0,374*
3128703	Sweet	4	0	<LOD	<LOD	<LOD	0,274*
3128703	Sweet	4	0	<LOD	<LOD	<LOD	0,285*
3128703	Sweet	5	0	<LOD	<LOD	<LOD	2,055
3128703	Sweet	5	0	<LOD	<LOD	<LOD	1,827
3128703	Sweet	5	0	<LOD	<LOD	<LOD	2,689
3128703	Sweet	6	0	0,714*	2,234	<LOD	12,183
3128703	Sweet	6	0	<LOD	2,340	<LOD	11,060
3128703	Sweet	6	0	0,805*	1,856	<LOD	10,264
3128703	Sweet	7	0	1,298	0,000†	1,085	0,000†
3128703	Sweet	7	0	1,188	0,000†	1,111	0,000†
3128703	Sweet	7	0	1,151	0,000†	1,056	0,000†
3128703	Sweet	8	0	2,248	1,040	0,333	2,385
3128703	Sweet	8	0	2,365	1,010	0,365	2,317
3128703	Sweet	8	0	2,273	1,009	0,368	1,989
3128703	Sweet	9	0	<LOD	<LOD	<LOD	<LOD
3128703	Sweet	9	0	<LOD	<LOD	<LOD	<LOD
3128703	Sweet	9	0	<LOD	<LOD	<LOD	<LOD
3128704	Menthol	1	0	0,000†	<LOD	0,000†	0,000†

Sample	Flavour	No Spiking					
		Lab Code	Nominal Spike level (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)	NNN (ng/g)
3128704	Menthol	1	0	0,000†	<LOD	0,000†	0,000†
3128704	Menthol	1	0	0,000†	<LOD	0,000†	0,000†
3128704	Menthol	2	0	<LOD	<LOD	<LOD	<LOD
3128704	Menthol	2	0	<LOD	<LOD	<LOD	<LOD
3128704	Menthol	2	0	<LOD	<LOD	<LOD	<LOD
3128704	Menthol	3	0	0,421	0,082	1,423	0,547
3128704	Menthol	3	0	0,158*	0,025*	0,277	0,118*
3128704	Menthol	3	0	0,175*	0,031*	0,257	0,119*
3128704	Menthol	4	0	<LOD	<LOD	<LOD	0,269*
3128704	Menthol	4	0	<LOD	<LOD	<LOD	0,263*
3128704	Menthol	4	0	<LOD	<LOD	<LOD	0,297*
3128704	Menthol	5	0	<LOD	<LOD	<LOD	1,364
3128704	Menthol	5	0	<LOD	<LOD	<LOD	1,364
3128704	Menthol	5	0	<LOD	<LOD	<LOD	1,372
3128704	Menthol	6	0	0,468*	3,769	0,679*	12,921
3128704	Menthol	6	0	<LOD	3,713	0,785*	13,946
3128704	Menthol	6	0	<LOD	3,571	<LOD	13,959
3128704	Menthol	7	0	1,198	0,000†	1,198	0,000†
3128704	Menthol	7	0	1,211	0,000†	1,150	0,000†
3128704	Menthol	7	0	1,214	0,000†	1,191	0,000†
3128704	Menthol	8	0	2,411	1,030	0,233*	2,055
3128704	Menthol	8	0	2,314	1,354	0,184*	2,119
3128704	Menthol	8	0	2,387	1,019	0,184*	2,726
3128704	Menthol	9	0	<LOD	<LOD	<LOD	<LOD
3128704	Menthol	9	0	<LOD	<LOD	<LOD	<LOD
3128704	Menthol	9	0	<LOD	<LOD	<LOD	<LOD

<LOD indicates that the value is below the limit of detection

The (*) symbol indicates the value is below the limit of quantitation, but above the limit of detection

The (†) symbol indicates the participating lab indicated the result was below the limit of quantitation, above the limit of detection, but did not report a result.

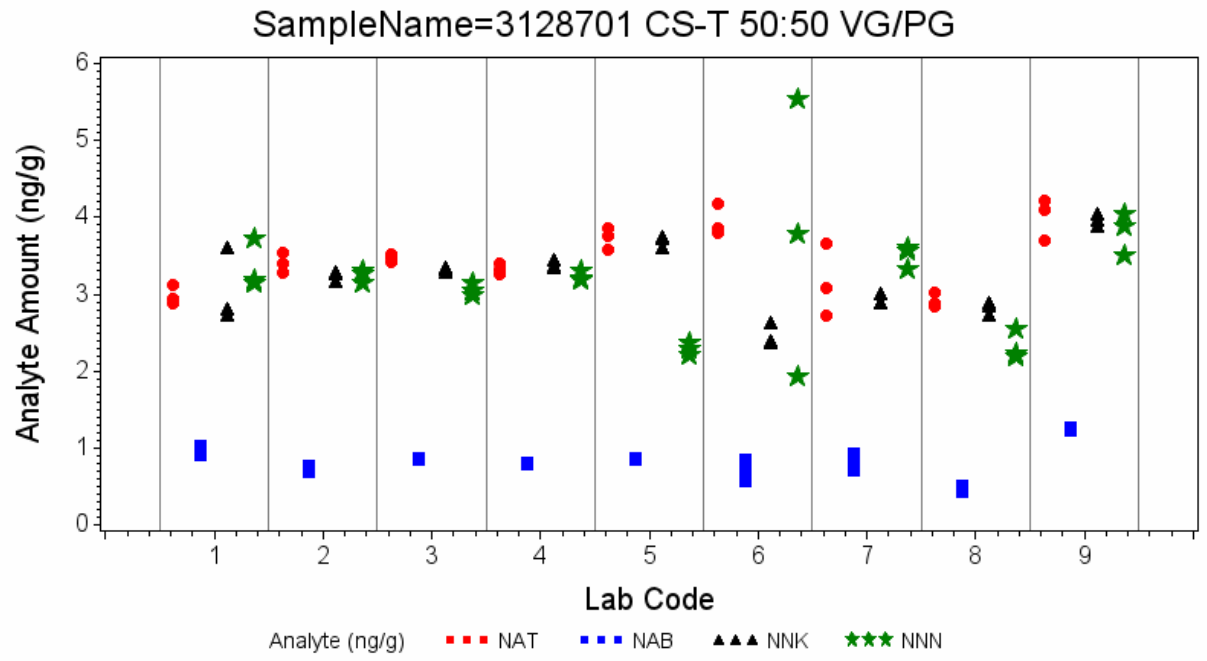
Sample	Flavour	Nominal Conc, (NAT, NNK, NNN/NAB) = 3,2/0,8 ng/g					
		Lab Code	Nominal Spike level (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)	NNN (ng/g)
3128701	Unflavoured	1	3,2/0,8	2,939	0,9083	3,614	3,156
3128701	Unflavoured	1	3,2/0,8	2,883	1,026	2,809	3,200
3128701	Unflavoured	1	3,2/0,8	3,133	1,015	2,735	3,730
3128701	Unflavoured	2	3,2/0,8	3,533	0,7043	3,266	3,161
3128701	Unflavoured	2	3,2/0,8	3,290	0,7008	3,166	3,280
3128701	Unflavoured	2	3,2/0,8	3,395	0,7667	3,286	3,318
3128701	Unflavoured	3	3,2/0,8	3,520	0,8737	3,320	3,070
3128701	Unflavoured	3	3,2/0,8	3,425	0,8625	3,351	3,006
3128701	Unflavoured	3	3,2/0,8	3,484	0,8462	3,285	3,161
3128701	Unflavoured	4	3,2/0,8	3,330	0,7982	3,349	3,207
3128701	Unflavoured	4	3,2/0,8	3,260	0,7984	3,376	3,218
3128701	Unflavoured	4	3,2/0,8	3,402	0,8229	3,454	3,321
3128701	Unflavoured	5	3,2/0,8	3,756	0,8715	3,756	2,385
3128701	Unflavoured	5	3,2/0,8	3,583	0,8787	3,613	2,220
3128701	Unflavoured	5	3,2/0,8	3,857	0,8469	3,733	2,301
3128701	Unflavoured	6	3,2/0,8	3,810	0,8464	2,381	3,789
3128701	Unflavoured	6	3,2/0,8	4,177	0,7109	2,390	5,550
3128701	Unflavoured	6	3,2/0,8	3,853	0,5807	2,632	1,942
3128701	Unflavoured	7	3,2/0,8	3,091	0,7115	2,889	3,335
3128701	Unflavoured	7	3,2/0,8	2,723	0,7718	2,898	3,616
3128701	Unflavoured	7	3,2/0,8	3,655	0,9307	3,004	3,580
3128701	Unflavoured	8	3,2/0,8	2,886	0,4436	2,842	2,236
3128701	Unflavoured	8	3,2/0,8	3,016	0,5183	2,883	2,570
3128701	Unflavoured	8	3,2/0,8	2,850	0,4685	2,733	2,205
3128701	Unflavoured	9	3,2/0,8	4,099	1,276	3,878	3,515
3128701	Unflavoured	9	3,2/0,8	3,705	1,238	4,047	3,900
3128701	Unflavoured	9	3,2/0,8	4,213	1,275	3,959	4,056

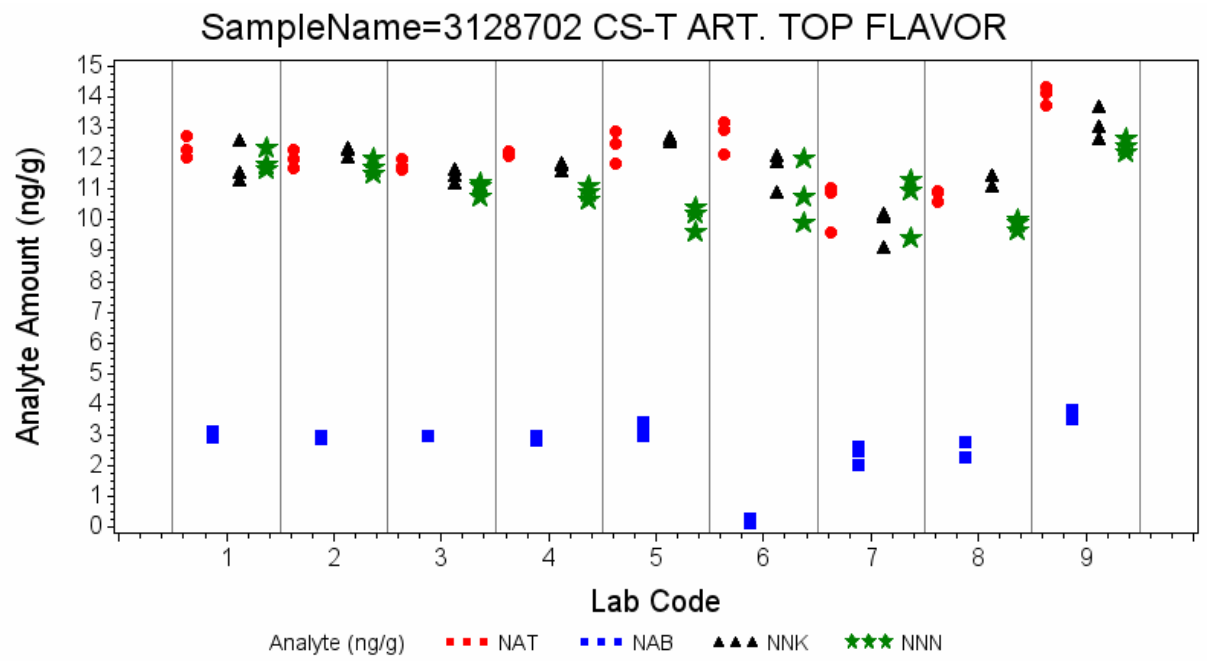
Sample	Flavour	Nominal Conc, (NAT, NNK, NNN/NAB) = 12/3 ng/g					
		Lab Code	Nominal Spike level (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)	NNN (ng/g)
3128702	Tobacco	1	12/3	12,31	3,139	12,58	11,85
3128702	Tobacco	1	12/3	12,06	2,909	11,54	11,68
3128702	Tobacco	1	12/3	12,73	3,004	11,30	12,37
3128702	Tobacco	2	12/3	11,99	2,885	12,34	11,73
3128702	Tobacco	2	12/3	11,71	3,000	12,33	11,55
3128702	Tobacco	2	12/3	12,27	2,904	12,08	12,02
3128702	Tobacco	3	12/3	11,64	3,004	11,44	11,25
3128702	Tobacco	3	12/3	11,99	2,990	11,20	10,81
3128702	Tobacco	3	12/3	11,73	3,000	11,68	11,14
3128702	Tobacco	4	12/3	12,26	2,956	11,81	10,96
3128702	Tobacco	4	12/3	12,07	2,862	11,86	11,14
3128702	Tobacco	4	12/3	12,17	2,822	11,63	10,71
3128702	Tobacco	5	12/3	12,88	2,974	12,67	9,64
3128702	Tobacco	5	12/3	11,82	3,105	12,55	10,26
3128702	Tobacco	5	12/3	12,49	3,407	12,69	10,44
3128702	Tobacco	6	12/3	13,21	0,3174	11,90	12,01
3128702	Tobacco	6	12/3	12,15	0,1562	12,12	9,932
3128702	Tobacco	6	12/3	12,94	0,2937	10,92	10,80
3128702	Tobacco	7	12/3	11,07	2,610	10,20	10,97
3128702	Tobacco	7	12/3	10,90	2,461	10,11	11,36
3128702	Tobacco	7	12/3	9,59	2,023	9,12	9,44
3128702	Tobacco	8	12/3	10,95	2,785	11,11	10,02
3128702	Tobacco	8	12/3	10,59	2,282	11,48	9,69
3128702	Tobacco	8	12/3	10,92	2,760	11,44	9,95
3128702	Tobacco	9	12/3	13,75	3,534	12,66	12,22
3128702	Tobacco	9	12/3	14,31	3,657	13,70	12,69
3128702	Tobacco	9	12/3	14,11	3,841	13,06	12,42

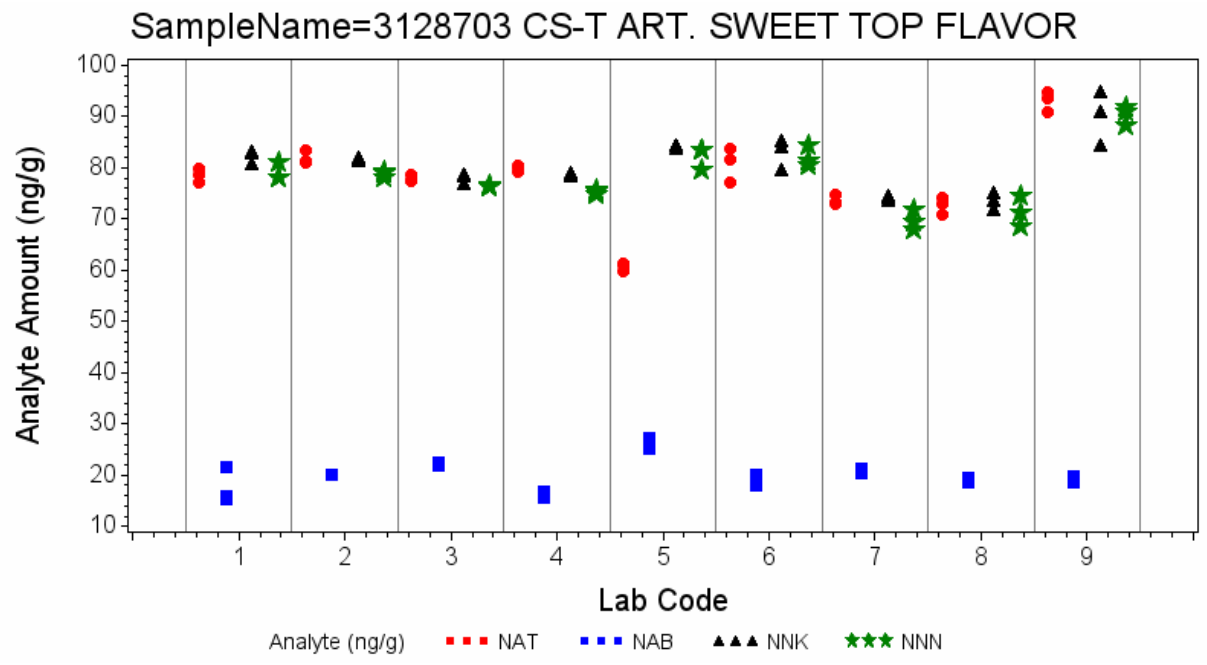
Sample	Flavour	Nominal Conc, (NAT, NNK, NNN/NAB) = 80/20 ng/g					
		Lab Code	Nominal Spike level (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)	NNN (ng/g)
3128703	Sweet	1	80/20	79,88	15,81	83,02	81,33
3128703	Sweet	1	80/20	77,24	15,42	83,09	78,18
3128703	Sweet	1	80/20	78,75	21,54	80,98	78,25
3128703	Sweet	2	80/20	81,42	20,08	81,47	79,20
3128703	Sweet	2	80/20	81,18	20,26	82,02	78,38
3128703	Sweet	2	80/20	83,43	20,21	81,50	79,42
3128703	Sweet	3	80/20	77,38	21,87	78,58	76,91
3128703	Sweet	3	80/20	77,35	22,53	78,87	76,45
3128703	Sweet	3	80/20	78,53	22,15	76,98	76,55
3128703	Sweet	4	80/20	80,42	16,85	78,98	75,78
3128703	Sweet	4	80/20	79,20	15,53	78,75	74,89
3128703	Sweet	4	80/20	80,06	16,19	78,57	75,51
3128703	Sweet	5	80/20	61,25	26,70	83,88	79,78
3128703	Sweet	5	80/20	59,94	27,37	84,40	83,59
3128703	Sweet	5	80/20	60,98	25,28	84,48	83,77
3128703	Sweet	6	80/20	83,80	20,19	84,18	80,60
3128703	Sweet	6	80/20	77,05	18,07	79,74	84,53
3128703	Sweet	6	80/20	81,80	19,82	85,28	81,62
3128703	Sweet	7	80/20	73,13	21,28	74,62	72,03
3128703	Sweet	7	80/20	74,85	21,24	74,15	69,77
3128703	Sweet	7	80/20	73,29	20,49	73,81	68,06
3128703	Sweet	8	80/20	74,11	19,43	71,82	74,70
3128703	Sweet	8	80/20	70,93	18,66	73,80	68,88
3128703	Sweet	8	80/20	72,93	19,39	75,24	71,39
3128703	Sweet	9	80/20	94,88	19,72	90,98	91,93
3128703	Sweet	9	80/20	93,47	19,68	94,88	91,08
3128703	Sweet	9	80/20	90,97	18,50	84,44	88,51

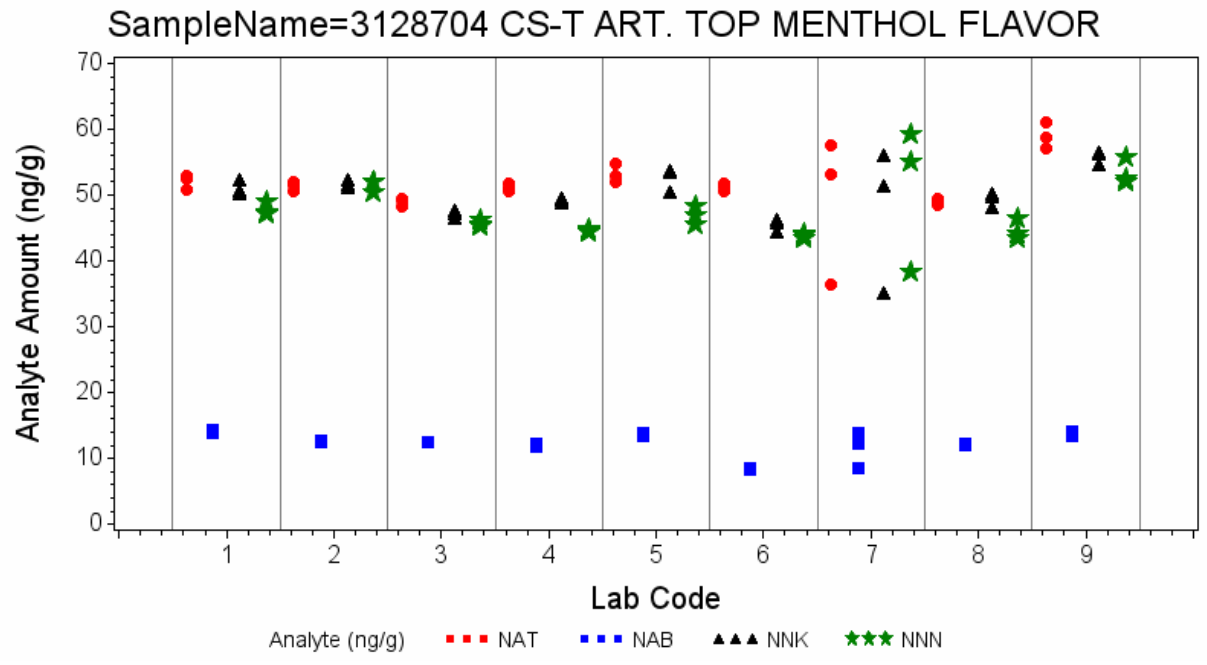
Sample	Flavour	Nominal Conc, (NAT, NNK, NNN/NAB) = 48/12 ng/g					
		Lab Code	Nominal Spike level (ng/g)	NAT (ng/g)	NAB (ng/g)	NNK (ng/g)	NNN (ng/g)
3128704	Menthol	1	48/12	50,79	14,37	50,79	47,34
3128704	Menthol	1	48/12	52,83	13,99	52,34	47,62
3128704	Menthol	1	48/12	52,38	14,01	50,19	49,21
3128704	Menthol	2	48/12	52,02	12,56	51,25	50,62
3128704	Menthol	2	48/12	51,55	12,80	52,42	52,16
3128704	Menthol	2	48/12	50,56	12,50	51,53	52,21
3128704	Menthol	3	48/12	49,24	12,52	47,75	45,54
3128704	Menthol	3	48/12	48,29	12,44	46,53	46,46
3128704	Menthol	3	48/12	49,40	12,59	47,23	45,79
3128704	Menthol	4	48/12	51,69	12,32	49,64	44,73
3128704	Menthol	4	48/12	51,10	11,82	48,77	44,93
3128704	Menthol	4	48/12	50,57	11,74	49,20	44,48
3128704	Menthol	5	48/12	54,89	13,42	53,53	48,45
3128704	Menthol	5	48/12	52,95	13,84	53,75	45,77
3128704	Menthol	5	48/12	51,99	13,77	50,41	47,07
3128704	Menthol	6	48/12	50,72	8,491	44,52	44,36
3128704	Menthol	6	48/12	51,84	8,246	45,82	43,61
3128704	Menthol	6	48/12	51,42	8,294	46,22	44,01
3128704	Menthol	7	48/12	36,44	8,664	35,17	38,38
3128704	Menthol	7	48/12	53,15	12,23	51,44	55,31
3128704	Menthol	7	48/12	57,67	13,83	56,12	59,41
3128704	Menthol	8	48/12	49,08	12,17	49,65	43,55
3128704	Menthol	8	48/12	49,53	12,02	48,19	44,25
3128704	Menthol	8	48/12	48,58	12,31	50,13	46,57
3128704	Menthol	9	48/12	61,15	14,07	56,55	55,93
3128704	Menthol	9	48/12	57,23	13,91	56,34	52,10
3128704	Menthol	9	48/12	58,69	13,41	54,75	52,78

APPENDIX D: Raw Data Plots






SampleName=3128703 CS-T ART. SWEET TOP FLAVOR
Analyte Amount (ng/g)
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
Lab Code
Analyte (ng/g)
NAT
NAB
NNK
NNN


SampleName=3128704 CS-T ART. TOP MENTHOL FLAVOR
Analyte Amount (ng/g)
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
Lab Code
Analyte (ng/g)
NAT
NAB
NNK
NNN