CORESTA Smokeless Tobacco Sub-Group

Working Group 2

2009 Collaborative Study Report

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List of Abbreviations

CDC	Centers for Disease Control and Prevention
CORESTA	Centre de Coopération pour les Recherches Scientifiques Relatives au Tabac
CRM	CORESTA recommended method
CSTS	CORESTA Smokeless Tobacco Sub-group
CV	Coefficient of Variation
ESTOC	European Smokeless Tobacco Council
GC-FID	Gas Chromatography with Flame Ionization Detection
GC-TCD	Gas Chromatography with Thermal Conductivity Detection
GC-TEA	Gas Chromatography with Thermal Energy Analysis
ISO	International Organisation for Standardisation
LC-MS/MS	Liquid chromatography with Tandem Mass Spectrometry
N	Number of laboratories
NAB	N-Nitrosoanabasine
NAT	N-Nitrosoanatabine
NNK	4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone
NNN	N-Nitrosonornicotine
r	repeatability
R	Reproducibility
SMNE	Swedish Match NE
TSNA	Tobacco Specific Nitrosamines

I – Summary

In 2009, a collaborative study involving 23 laboratories took place to assess the repeatability and reproducibility of a selection of methods used to determine the pH, water, nicotine, moisture and tobacco specific nitrosamines (TSNAs) in nine types of smokeless tobacco products. This collaborative study was coordinated by the Working Group 2 of the CORESTA Smokeless Tobacco Sub-group (CSTS).

The results were pooled and a statistical analysis according to ISO 5725-2 (1994) was performed. The CSTS discussed these results at a meeting in October 2009 and it was agreed that the results were generally consistent. It was decided that the methods used for pH and TSNAs could form new CORESTA recommended methods (CRMs) while the existing methods for nicotine and water (CRM 62 and 56 respectively) would be updated to reflect the findings of the collaborative study (i.e. extension of the scope and updates of the r and R values). For moisture content, a further collaborative study stipulating as many parameters as possible would be necessary to progress to a harmonised approach.

II – Background

Future tobacco product regulation is likely to require the reporting of constituent levels in smokeless tobacco. At present, there is a lack of international standards for the determination of constituents in smokeless products.

In 2007/08, the European Smokeless Tobacco Council (ESTOC) conducted a proficiency test to investigate the inter-laboratory variation in analytical techniques for the measurement of selected constituents, namely water, pH, nicotine, nitrate ion, N-nitroso-dimethyl amine, tobacco specific nitrosamines (TSNAs), benz(a)pyrene, aflatoxins, cadmium, lead, chromium, nickel and arsenic. The nine participating laboratories used their own validated methods, and consequently several different methods, instrumentations and work-up procedures were used for most of the analytes. Seven European and two American-style smokeless tobacco products were included in the study. The results for some analytes, such as nicotine and TSNAs, were consistent and the study group recommended that collaborative studies should be performed.

Following the formation of the CORESTA Smokeless Tobacco Sub-group (CSTS) at the end of 2008, the ESTOC Scientific Committee agreed to the transfer of collaborative studies to the CSTS. This approach brought the benefit of much wider industry participation, both in terms of products and markets.

The CSTS conducted an initial collaborative study in 2009 to compare laboratory data for water, pH, nicotine, moisture (oven volatiles) and TSNAs. In this study, a total of 23 participating laboratories included some from Europe, USA, Canada and Asia. The suite of test products consisted of four European, four American and one Asian-style smokeless tobacco product.

III – Project organisation

The project was undertaken by working group 2 of the CORESTA smokeless tobacco sub-group and was led by Linda Drake (British American Tobacco, U.K.). The protocol was designed and the samples coded by Linda Drake. The samples were kindly supplied by Pöschl Tabak, Fielder & Lundgren (British American Tobacco), Swedish Match, House of Oliver Twist, Conwood Company (later American Snuff Company), R.J.Reynolds Tobacco, I.T.C. and US Smokeless Tobacco. The US products were distributed by US Smokeless Tobacco and the European products and the Indian product were distributed by Swedish Match. The data were collated by Linda Drake and the statistics performed by Alexander Hauleithner (Japan Tobacco International, Austria).

IV – Participating laboratories

The following 23 laboratories took part in the study. They are listed alphabetically and the order in the list does not reflect the laboratory codes which are used to keep the results anonymous.

Arista Laboratories
Arnold Andre
British American Tobacco - GR&D
British American Tobacco - House of Prince
Borgwaldt ASL
CNTC
Conwood Company/American Snuff Company
Eurofins
Global Laboratories
ITC India
ITG Reemtsma
Japan Tobacco International Austria
KT&G
Labstat
Philip Morris International
Poeschl Tobak
R.J.Reynolds Tobacco
SEITA/Imperial Tobacco
Swedish Match NA
Swedish Match NE
Swisher
University of Kentucky (Dept of Plant & Soil Sciences)
US Smokeless Tobacco Manufacturing Company/Altria

V – Products

Nine smokeless tobacco products were tested representing major types of smokeless tobacco products used around the world. Table 1 summarises the sample code and product type.

TABLE 1. Sample Products

Sample code	Product description	Brand	Manufacturer	Market
1	Nasal snuff	Gletscher Prise	Poeschl Tabak	Europe
2	Loose snus	General	Swedish Match (NE)	Europe
3	Chewing tobacco – Bits*	Oliver Twist Tropical	House of Oliver Twist	Europe
4	Chewing tobacco-Flake	Gutkha Brand	Purchased by I.T.C. from the market	India
5	Pellet	Camel Orbs	R.J.Reynolds Tobacco	U.S.
6	Chewing tobacco – loose leaf	Redman	Swedish Match (NA)	U.S.
7	Moist snuff	Copenhagen Snuff	US Smokeless Tobacco Manufacturing Company	U.S.
8	Moist snuff	Grizzly Wintergreen Long Cut	Conwood/American Snuff	U.S.
9	Pouched snus	Mocca Mint	Fielder & Lundgren (British American Tobacco)	Europe

*Referred to as 'Chewing Tobacco – Twist' in the Study Protocol, Appendix 1

All samples except samples 3 and 5 were prepared by the manufacturers to give a homogenised batch of tobacco with a particle size ≤ 4 mm and supplied to the two distributors (US Smokeless Tobacco Company for the US and Swedish Match NE for Europe). Samples 3 and 5 were homogenised by the distributors. All samples were repackaged by the distributors into two plastic bags (one inside the other) to minimise the loss of volatiles and reduce cross contamination. The samples were sent frozen to each participating laboratory, but defrosting occurred during transport. Each laboratory was instructed to keep the samples frozen prior to analysis (scheduled over a defined 2-week period) and refrigerated between analyses. See protocol (Appendix 1) for more details.

VI – Analytes and recommended analytical methods

The analytes included in this survey were: nicotine, water (measured before and after analysis of other analytes), oven volatiles/moisture, pH and tobacco-specific nitrosamines (TSNAs) including

NNN, NNK, NAB and NAT. For each type of analyte, the following methods were recommended (see Appendix 1 for more details):

Water

The water content was measured both before and after analysis of other analytes, if possible, using CRM 56 (Karl Fisher) or CRM 57 (GC-TCD).

Moisture

Moisture content was measured using the participants' own in-house validated analytical procedures.

pH

The Centers for Disease Control and Prevention (CDC) method (Federal Register, Vol. 74, No. 4, 2009, pgs 712-718, see Appendix 2) was used with a recommended stirring time of 30 minutes and additional measurements at 5 and 15 minutes, where possible, to determine the most suitable length of time. Laboratory environmental conditions were also recorded.

Nicotine

CRM 62 (GC-FID) was used for the measurement of nicotine.

TSNAs

Laboratories with LC-MS/MS capability used a method recommended by Swedish Match NE (see Appendix 3). Other laboratories used alternative technologies such as GC-TEA. Because there is reportedly some variability in TSNA standard material used by different laboratories, a TSNA calibration standard was distributed by Swedish Match NE, along with the samples, in order to normalise the results if necessary. Laboratories were requested to analyse this standard as a sample against their in-house calibrations and to include the results along with those of the test samples. Table 2 summarises the number of laboratories that followed the recommended methods for each analyte (except moisture as there was no recommended method).

All analyses were performed with 3 replicates (including both sets of analysis for water) and individual results were submitted. Also, a description of the analytical procedure used to analyse the samples, including sample sizes, extraction techniques, detection limits, quantification limits and other relevant information were to be entered into a spreadsheet provided and sent together with the collected analytical data.

All results are summarised in the full statistical report (Appendix 4 - p 72 to 77) as a mean and standard deviation for each analyte, each sample and each laboratory, alongside the method used by the laboratory for the different analytes.

TABLE 2. The Number of Laboratories Using the Various Methods

Analytes	Recommended	Other	Not analysed
Water	KF (CRM 56): 4 labs GC (CRM 57): 5 labs	KF (other): 8 labs GC (other): 2 labs NIR: 1 lab	3 labs
pH	CDC: 12 labs	CDC? 8 labs Other : 1 lab	2 labs
Nicotine	CRM 62: 5 labs	CDC: 5 labs Own: 9 labs CDC?: 2 labs ?: 2labs	none
TSNA	LC-MS/MS SMNE: 9 labs	LC-MS/MS: 3 labs GC-TEA: 6 labs GC-NCD: 1 lab	4 labs

** The question marks in Table 2 illustrate the fact that it was not possible to confirm that the laboratory had been following a particular method.*

VII – Statistical analysis

The statistical analysis was performed by Alexander Hauleithner, JTI-Ökolab (Austria). As per ISO 5725-2, data consistency was checked using graphical and numerical outlier detection techniques as summarised in Table 3.

TABLE 3. Outlier Detection Techniques

Data consistency	**Graphical**	**Numerical**
Inter-laboratory	Mandel's *h*	Grubbs' single outlier
Intra-laboratory	Mandel's *k*	Cochran's C test

Overall data analysis per sample type

These tests were applied to all the data, regardless of the technique used, in the first instance. All outliers and stragglers detected are displayed in Table 5, pages 23-24 of Appendix 4. Table 4 below summarises the number of laboratories found to be outliers for the intra-laboratory and inter-laboratory consistency of their data.

TABLE 4. Number of Outlier Laboratories per Analysis per Sample

Analytes	Water before	Moisture	pH	Nicotine	TSNA sum	Water after
Number of participating laboratories	18	20	21	23	18 or 19	20
Sample 1	2	1	4	3	3	1
Sample 2	2	0	4	6	3 *	2
Sample 3	2	0	3	3	0	2
Sample 4	4	0	2	2	2 *	3
Sample 5	2	0	4	3	3 *	2
Sample 6	1	0	1	6	2 *	2
Sample 7	2	0	3	3	0	2
Sample 8	2	0	5	3	0	5
Sample 9	1	1	4	3	0	4

* Denotes 18 participating laboratories

The rest of the data were used to calculate the mean, repeatability (r), reproducibility (R) as well as their respective standard deviations (r SD and R SD) and coefficient of variation (CV r and CV R) for each analyte and sample type. The r and R was calculated for results reported by at least 3 laboratories. The data are displayed in Tables 6-14 on pages 24-25 of Appendix 4.

Data analysis grouped by analytical methods

The results for the water analysis, nicotine analysis and pH determination were then sorted depending on the technique used as follow:

- Water results: GC (CRM 57) and KF (CRM 56)
- Nicotine results: CRM 62, CDC and OWN
- pH results: CDC and CDC?

The outlier detection was performed on the separate data set. After removal of the outliers, the mean, repeatability (r), reproducibility (R) as well as their respective standard deviations (r SD and R SD) and coefficient of variation (CV r and CV R) for each analyte, sample type and technique were calculated. Tables summarising these results can be found on pages 26-29 of Appendix 4.

The total TSNAs and individual TSNAs (NNN, NAT, NAB and NNK) were analysed in two different ways: as received (results as received with no correction factor applied) and corrected (i.e. corrected using the results received for the calibration standard which was distributed to each laboratory together with the samples). The results were grouped by technique: LC-MS/MS SMNE (recommended method), LC-MS/MS and GC and an outlier detection and removal were performed

on each data set. The full results can be found in Appendix 4, on pages 30-37 for the "as received" and on pages 37-41 for "corrected".

VIII –Comments on the results

Overall data analysis

Sample 4 (chewing tobacco flakes) gave the most variable results for both intra- and inter-laboratory comparisons (highest CV r and CV R). This type of sample may prove harder to homogenise and/or analyse. Nevertheless, all CV r for all analytes were below 10% and therefore good. The CV R values were mainly between 10 and 23% which is satisfactory.

When looking at the analytes, the analysis of TSNA gave the most variable inter-laboratory results. No CV R was below 10%. The CV R were all between 10 and 23% which is still satisfactory.

The CV R for pH on every type of sample was below 2%. This indicates a very good agreement between the participating laboratories (N=16 to 20).

Results by analysis and method

To facilitate the discussion of the results, Tables summarising the CV r and CV R was created for each type of analyte and method (Tables 5 to 9). To prevent a biased interpretation and outline the possible influence of sample matrix on the data variability for some of the samples, any value that was particularly different from others was stated in the comments box alongside the sample number it was relating to. Usually, a CV value below 10% is excellent, 10 to 30% is satisfactory and above 30% is unsatisfactory. N, the number of laboratories, is expressed as a range as it varied across sample types.

Water analysis

The CV r and CV R for the water results (before and after analysis of other analytes) are shown in Table 5 for all methods combined, the GC (CRM 57) and KF methods (CRM 56).

TABLE 5. Water Analyses

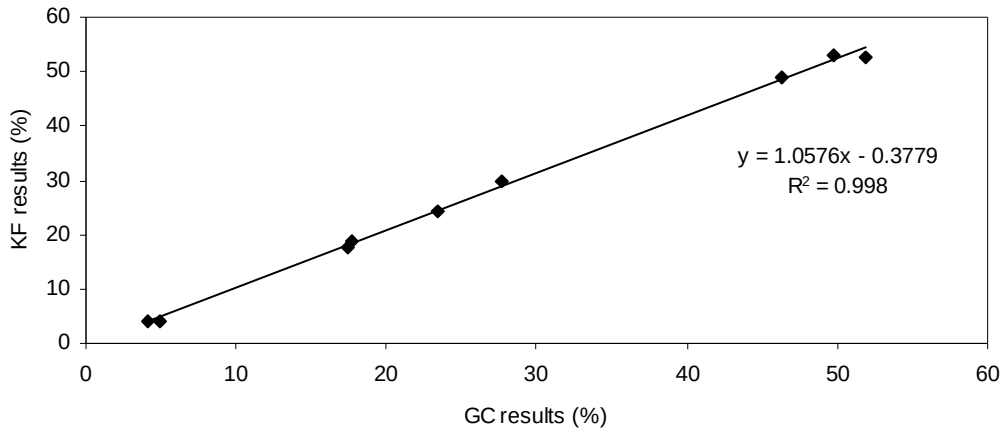
Analyte	N	CV r	CV R	Comments
Water Before	14-17	≤3.0%	≤7.5%*	* 18.2% sample 4, 20.1% sample 5
Water GC Before	5-6	≤1.7%	≤16.4%	None
Water KF Before	10-11	≤3.7%*	≤6.3%**	* 7% sample 4; **17.8% sample 4, 23.9% sample 5
Water After	15-18	≤3.6%	≤10.7%*	* 18.0% sample 4, 13.5% sample 5
Water GC After	6-7	≤3.6%	≤17.2%*	* 22.5% sample 4
Water KF After	9-11	≤3.6%	≤7.8%*	* 13.6% sample 4

In this collaborative study, the results from the Karl Fisher method showed slightly less variability across laboratories and the CV R were good. The CV R values for the GC method were satisfactory overall. The "after analysis" was more variable; possibly due to the fact that samples were kept in slightly different conditions from one laboratory to another and therefore the loss of water occurring would be uneven and add variability to the results. Samples 4 and 5 (Flakes and Pellets respectively) showed higher CV R with both methods. However, these types of samples had the lowest water content (about 4%) which inflates the CV (ratio of the SD over the mean).

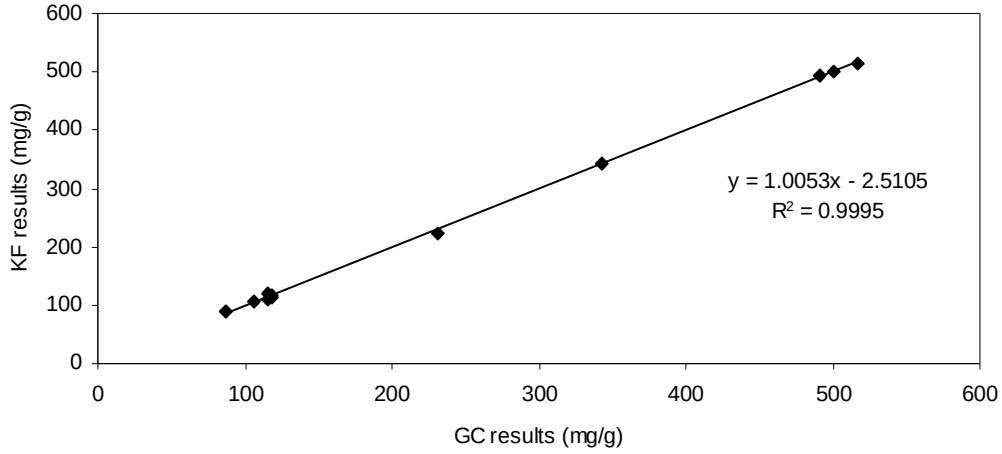
The current CRM 56 and 57 are based on a CORESTA collaborative study in December 2002 involving 17 laboratories. In that study, the CV R were lower for both methods but showed a similar trend in the sense that the variation in the KF results was also tighter across laboratories. In the 2002 collaborative study, the water content of the test products ranged from approx. 9% to 52% and both current CRMs state that the methods can be used for products ranging from 2 to 55% water content on a weight by weight basis. Interestingly the highest CV R values observed in the 2009 collaborative study were for the products which had a low water content of approx. 4 to 5 %. Those products, or products of similar water content, were not tested in 2002. Hence the low water content of some products may contribute to the relatively higher CV values observed in this study.

Graph 1 shows a correlation plot of the GC and KF results (before results only). The slope of 1.0576 seems to indicate a slight bias of the KF results compared to the GC, the KF results being about 6% higher. The 2002 CORESTA collaborative study showed that there was no difference between the two methods. For comparison, Graph 2 shows the correlation between the GC and KF results for that previous collaborative study. The slope for the correlation was then 1.0053, indicating a bias of about 0.5%. This bias is negligible compared to the inter-laboratory variation (CV R of about 9% and 5% for the GC and KF results respectively).

Graph 1: Correlation between GC and KF results for water analysis (2009)



Graph 2: Correlation between GC and KF results for water analysis (2002)



In both studies, the GC method yielded a slightly higher CV R than the KF method indicating that KF method is more reproducible and/or precise. In 2002, both the CV r and CV R for the GC method tended to be higher than for the KF method. In 2009, the CV R for the GC method tended to be higher overall, but the KF method gave higher CV R values for the driest products (chewing flakes (sample 4) and pellets (sample 5)) indicating less reproducibility than the GC method for those types of products. Overall the GC method may be better for precision and reproducibility if products of lower water content are to be analysed on a regular basis.

Moisture

For moisture, no method was recommended and each laboratory used its own in-house validated method. The CV r and CV R results (N = 19-20) were all relatively low (≤2.3% and ≤11.4% respectively) with all chewing tobaccos (bits, flakes and loose leaf) and the pellets displaying the highest CV R (between 5.0 and 11.4%) compared to the other types of sample. However, it should be noted that the Flakes and Pellets moisture content was relatively low (5 to 6%) compared to the other types of product (20 to 60%) which influences CV R. Despite the absence of a general method to follow, the agreement between the participating laboratories was good.

pH

The CV r and CV R for the pH results are shown in Table 6 for all methods combined, CDC and 'CDC?'. Out of the 21 laboratories which provided data for pH, 12 confirmed the use of the CDC method. After removal of outliers for each sample type (maximum 2), the CV r were all below 0.5% and the CV R below 2%. This method provided very consistent intra- and inter-laboratory results.

TABLE 6. pH analysis

Analyte	N	CV r	CV R	Comments
pH all	16-20	≤0.4%	≤1.9%	None
pH CDC	10-12	≤0.4%	≤1.8%	None
pH CDC?	6-8	≤1.1%	≤4.5%	None

In the protocol, it was recommended that the pH of the nine samples was to be measured after 5, 15 and 30 minutes extraction time. The length of extraction time showed no major influence on the results (see page 30 of Appendix 4).

Nicotine

The CV r and CV R for the nicotine results are shown in Table 7 for all methods combined, CRM 62, CDC and the laboratories' own method (OWN).

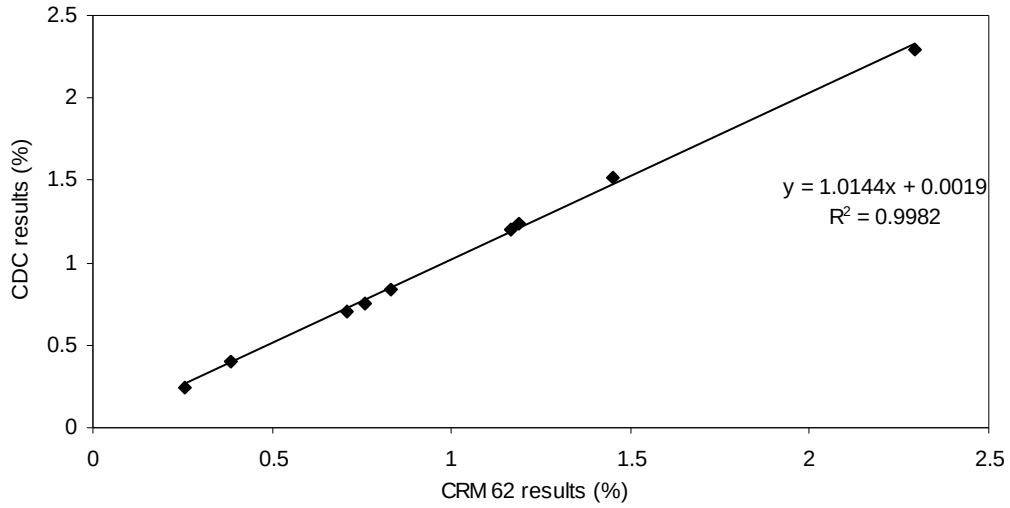
TABLE 7. Nicotine Analyses

Analyte	N	CV r	CV R	Comments
Nicotine all	17-21	≤5.8%	≤13.4%	None
Nicotine CRM 62	3-4	≤4.3%	≤10%*	* 13.3% sample 5, 15.8% sample 4
Nicotine CDC	5-7	≤5.5%	≤8.6%*	* 12.5% sample 5, 16.9% sample 4
Nicotine OWN	6-8	≤6.6%	≤11%*	* 15.2% sample 5

The inter-laboratory variation for both the CRM 62 and CDC methods was relatively low. Sample 4 and 5 (Flakes and Pellets) proved more variable regardless of the methods, suggesting that it may be due to the product heterogeneity or the difficulty of homogenising samples when preparing the samples for analysis or performing the analytical procedures. It is interesting to note that the CV r and CV R derived from the data stated in CRM 62 (≤3.6% and ≤6.4% respectively) are reasonably consistent with the results from this collaborative study (excluding sample 4 and 5).

Graph 3 illustrates the correlation between the results obtained using the CDC and the CRM 62 methods. The correlation is excellent and the bias negligible (<1.5%) compared to the CV R for those methods (about 10%).

Graph3: Correlation between CRM 62 and CDC results for nicotine analysis



One of the participating laboratories reported that one sample type showed interferences and that standard addition should be tested and used if necessary as recommended in the CDC method.

Tobacco Specific Nitrosamines

The recommended method for the analysis of these compounds was the LC-MS/MS SMNE method.

TSNA sum (sum of the four individual TSNA)

Table 8 summarises the CV r and CV R for the TSNA sum results for all methods (all), LC-MS/MS SMNE (SMNE), LC-MS/MS (LC) and GC-TEA (GC) "as received" and "corrected" (corr.).

TABLE 8. TSNA sum analysis

Analyte	N	CV r	CV R	Comments
TSNA sum all	15-19	≤7.3%	≤22.2%	None
TSNA sum SMNE	6-9	≤6.6%	≤13.3%*	* 21.6% sample 3
TSNA sum LC	3	≤8.4%*	≤16.7%**	* 17.4% sample 6, **21.7% sample 2, 29.9% sample 6, No values for sample 5
TSNA sum GC	5-7	≤7.7%	≤39.9%	None
TSNA sum all corr.	11-15	≤7.2%	≤24.9%	None
TSNA sum SMNE corr.	8-9	≤6.6%	≤28.2%	None
TSNA sum LC corr.	3	≤8.0%*	≤30.2%	* 17.5% sample 6
TSNA sum GC corr.	3	≤10%	≤11.2%*	* 21.1% sample 3, 25.3% sample 4 No values for sample 2

In Table 8, N can differ between the "as received" and "corrected" data because the outliers were tested on each set of data separately (e.g. a laboratory which was an outlier "as received" was still included "corrected" set and could then be deemed as not being an outlier in that new set after correction).

The CV r and CV R were much higher than for the other analytes. This is most likely due to the fact that the levels of TSNA in the samples are lower than the rest of the analytes measured in this collaborative study. The analysis of trace compounds is inherently associated with a higher uncertainty around the measurements, heavily contributing to the intra- and inter-laboratory variability.

Applying a correction factor to compensate for the reportedly variable reference material affected the reproducibility in a different way depending on the methods.

The CV R for the sum remained similar but the correction dramatically improved the reproducibility between the laboratories which used the GC-TEA method. But this improvement could be an artefact of the way these results were calculated as the number of laboratories included in the calculation fell from 5-7 to 3; a direct comparison of "as received" versus "corrected" should be considered with caution as the laboratories which did not provide data for the unknown standard were not included in the "corrected" data set but still included in the "as received" data set. In order to correctly assess the impact of correction on the data spread it would be necessary to perform further statistics on both data sets for the same laboratories, all of whom provided results for the unknown standard. For the two LC-MS/MS methods, the CV R almost doubled which means that there was a lot of variability introduced by applying the correction factor. This is against expectation as the correction should remove the variability introduced by the use of a variable reference material. The number of laboratories taken into account before and after correction is similar in this case and should not have influenced the change. Because the CV R was below 30% after correction, the inter-laboratory variability for the recommended method (SMNE) is still satisfactory.

N-Nitrosonornicotine (NNN)

Table 9 summarises the CV r and CV R for the NNN results for all methods (all), LC-MS/MS SMNE (SMNE), LC-MS/MS (LC) and GC-TEA (GC) as received and corrected (corr.).

The correction factor applied to the results had no significant influence on the sum but, yet again, decreased the variation for the GC results. For the latter, the number of laboratories taken into account decreased from 6-7 to 3 and it is therefore difficult to draw any conclusion.

For the LC-MS/MS method, the results were affected in both directions resulting in a wider spread of the CV R values across sample types.

For the SMNE method, the CV R results increased for all samples, rising from approx.10-15% to 20-25% on average. Although the inter-laboratory variability is quite large, it is still satisfactory.

TABLE 9. NNN analysis

Analyte	N	CV r	CV R	Comments
NNN all	17-19	≤8.0%	≤26.9%	None
NNN SMNE	8-9	≤7.9%	≤17%*	* 22.9% sample 5
NNN LC	3	≤11.8%*	≤38.2%	* 20.4% sample 6
NNN GC	6-7	≤8.6%	≤29.2%	None
NNN all corr.	13-15	≤7.6%	≤27.3%	None
NNN.SMNE corr.	8-9	≤7.2%	≤27.5%	None
NNN.LC corr.	3	≤11.3%*	≤27.3%**	* 21.5% sample 6 **42.9% sample 5
NNN.GC corr.	3	≤8.6%*	≤13.1%**	* 18.3% sample 2; ** 26.7% sample 2 No values for sample 7

N-Nitrosoanatabine (NAT)

Table 10 summarises the CV r and CV R for the NAT results for all methods (all), LC-MS/MS SMNE (SMNE), LC-MS/MS (LC) and GC-TEA (GC) as received and corrected (corr.).

TABLE 10. NAT analysis

Analyte	N	CV r	CV R	Comments
NAT all	16-19	≤9.6%	≤37.6%	None
NAT SMNE	7-9	≤9.3%	≤29.6%*	* 36.4% sample 3
NAT LC	3	≤8.7%*	≤21.0%**	* 15.6% sample 5, 17.2% sample 6 ** 36.6% sample 6
NAT GC	5-7	≤11.1%	≤46.1%	None
NAT all corr.	13-15	≤8.8%	≤29.1%	None
NAT corr. SMNE	7-9	≤8.5%	≤27.9%*	* 33.4% sample 3
NAT corr. LC	3	≤16.6%	≤25.4%*	* 37.4 % sample 6
NAT corr. GC	3	≤11.0%	≤26.5%*	* 40.0 % sample 4, 41% sample 2 No values for samples 1 and 9

The correction factor brought all CV R values down for the sum and the SMNE method. The GC and LC-MS/MS methods were affected in both directions depending on the sample increasing the spread of the CV R across the samples.

The GC method produced the most variable results whilst the recommended method (SMNE) produced the most consistent results across laboratories. The inter-laboratory variability for the latter was high but still satisfactory (apart for sample 3).

N-Nitrosoanabasine (NAB)

Table 11 summarises the CV r and CV R for the NAB results for all methods (all), LC-MS/MS SMNE (SMNE), LC-MS/MS (LC) and GC-TEA (GC) as received and corrected (corr.).

TABLE 11. NAB analysis

Analyte	N	CV r	CV R	Comments
NAB all	9-15	≤11.0%	≤23.7%*	* 32.1% for sample 5
NAB SMNE	8	≤11.3%	≤18.9%*	* 25.1% sample 5
NAB LC	3	≤12.0%	≤69.9%	Extremely high inter-laboratory variation No values for samples 3 and 6
NAB all corr.	9-13	≤11.5%	≤29.5%*	* 39.8% for sample 5
NAB SMNE corr.	8	≤11.7%	≤30.5%	None
NAB LC corr.	3	≤12.1%	≤77.1%	Very high inter-laboratory variation except for samples 7 and 8 (<10%). No values for samples 3 and 6

The CV R for the GC method are not listed in the Table as they were only provided for sample 7 (20.0%) and 8 (33.1%) before correction and none after.

After application of the correction factor, the CV R values for both LC-MS/MS methods increased. For the non-SMNE LC-MS/MS method, the number of CV R above 30% (unsatisfactory) moved from 3 to 4 out of 7 samples indicating that the method did not produce reproducible results overall. Although the CV R for the SMNE method jumped from12-20% to 20-25% after correction for most of the samples, the reproducibility between laboratories was satisfactory overall (only sample 5 had a CV R of 30.5% but that sample had one of the lowest level).

4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK)

Table 12 summarises the CV r and CV R for the NNK results for all methods (all), LC-MS/MS SMNE (SMNE), LC-MS/MS (LC) and GC-TEA (GC) as received and corrected (corr.).

TABLE 12. NNK analysis

Analyte	N	CV r	CV R	Comments
NNK all	13-18	≤13.5%	≤19.7%	
NNK SMNE	7-9	≤7.1%	≤14.2%*	* 22.8% sample 8, 24.7% sample 7
NNK LC	3	≤10.2%*	≤21.5%**	* 15.2% sample 6, 19.2% sample 3 ** 30.1% sample 5
NNK GC	3-6	≤10.1%*	≤18.5%**	* 17.8% sample 3 ** 23.1% sample 2, 23.6% sample 3, No values for sample 4
NNK all corr.	12-15	≤15.0%	≤25.2%	
NNK SMNE corr.	8	≤7.1%	≤26.2%	
NNK LC corr.	3	≤10.0%*	≤17.6%**	* 15.7% sample 6, 20.1% sample 3 ** 26.4% sample 3, 33.7% sample 5
NNK GC corr.	3	≤7.3%	≤34.3%*	* 75.7% sample 2. No values for samples 3, 4, 5 and 6

After application of the correction factor, the CV R values for the combined methods, the GC and the SMNE method all increased while for the LC-MS/MS method, they mainly decreased. Nevertheless, the SMNE method produced the most consistent results across laboratories overall by as all CV R were below 30% and therefore satisfactory compared to the other two methods.

Conclusion for the TSNA analysis

Overall, the recommended method (LC-MS/MS SMNE) produced the most consistent results during this collaborative study. It should be noted that in most cases the correction factor, which was applied to compensate for the inconsistency found in the reference material used as a standard, introduced variability and this was unexpected.

Overview of the laboratory performances

Table 13 summarises the performance of each laboratory by using their absolute Z-score to categorise their results for each analyte. An absolute Z-score of 0 to 2 is good, 2 to 3 is satisfactory, 3 to 4 is questionable and > 4 is unsatisfactory. Note that for TSNA, only the Z-score on corrected data are stated here. The full results can be found in Appendix 4, pages 56-71.

TABLE 13. Laboratory Performance Summary

	Laboratory Number by Performance Category			
Analyte	Good	Satisfactory	Questionable	Unsatisfactory
Water Before	1, 2, 7, 8, 9, 10, 12, 13, 14, 16, 17, 19, 22, 23	No lab	11	3, 18, 20
Water After	6, 7, 8, 11, 12, 13, 14, 15, 16, 17, 19, 22, 23	1, 2, 20	9	3, 18
Moisture	1, 2, 4, 6, 7, 8, 9, 12, 13, 20, 23	14, 15, 21	3, 17, 22	5, 10, 19
pH	1, 3, 5, 6, 7, 10, 12, 13, 15, 16, 19, 20, 21 22, 23	2, 4, 18	9, 14	8
Nicotine	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 17, 19, 21, 22, 23	4, 15, 20	18	16
TSNA sum corr.	1, 2, 3, 6, 7, 8, 9, 10, 12, 14, 17	No lab	4, 13, 21	23
NNN corr.	1, 2, 3, 6, 8, 9, 10, 12, 14, 17, 21	7, 13	No lab	4, 23
NAT corr.	1, 2, 3, 6, 7, 8, 9, 10, 12, 14, 21	4	13, 17	23
NAB corr.	1, 2, 3, 7, 8, 10, 13, 14	4, 12, 17	No lab	9, 23
NNK corr.	1, 3, 4, 7, 8, 9, 10, 14, 17	2, 6, 12, 13	21	23

IX –Recommendations

After reviewing the statistical results, the CSTS recommended the following:

1. A new CRM should be written for the pH analysis, reflecting the recommended method used for this study.
2. The CRM for both nicotine and water (CRM 62 and 56 respectively) should be amended to extend the scope of application and update the r & R to reflect the findings of this study.
3. A new CRM should be written for TSNA based on the method used in this study.
4. A method stating standardised parameters should be written for moisture and used in the next collaborative study planned for Q2, 2010.

CSTS Collaborative Study 2009
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Appendix 1

CORESTA Smokeless Tobacco Sub Group

Proposed 2009 Collaborative Study – Draft

Linda Drake (BAT) Study Coordinator

Confidentiality Notice: All data in this Collaborative Study should be handled in the strictest of confidence by all participating laboratories.

1. Introduction

In November 2008, the CORESTA Scientific Commission recommended that a smokeless tobacco sub group be established. At the inaugural meeting of this sub group, a working group was set up with the objective of designing and conducting a collaborative study for pH, water, moisture, nicotine and TSNAs during 2009. The initial objective for the sub group was to design an agreed protocol.

2. Objective

This study has been proposed to investigate both 'within' and 'between' laboratory variation in analytical techniques for the measurement of each of the proposed analytes & product characteristics. These are:-

pH
Water
Moisture
Nicotine
TSNAs

3. Time schedule

Action	By whom	May	June	July	August	September	October
Confirm participating laboratories and request despatch/import information	LD	22nd					
Confirm suppliers' ability to process and despatch 5kg sample to distributors (POESCHL, CONWOOD, RJRT, ITC, SMNE, UST)	LD/suppliers	22nd					
Revise protocol and spreadsheet. Circulate to sub group	LD		26th				
Despatch 5kg samples to distributors	suppliers		26th				
Homogenised, suitably labelled samples despatched to labs	distributors			10th			
Analysis	labs			27th	7th		
Data on spreadsheet submitted to LD	labs				14th		
Prepare and code data, submit to AH	LD				21st		
Prepare draft statistical report	AH					18th	
Circulate report to sub group	LD					21st	
Present outcomes at next meeting	LD						1st

LD – Linda Drake, BAT, Southampton
AH – Alexander Hauleithner, JTI, Austria

4. Participating Laboratories

The following 23 laboratories have kindly agreed to take part in the study:-

Arista Laboratories
Arnold Andre
BAT GR&D
BAT House of Prince
Borgwaldt ASL
CNTC
Conwood
Dept of Plant & Soil Sciences, University of Kentucky
Eurofins
Global Laboratories
ITC India
ITG Reemtsma
JTI Austria
KT&G
Labstat
PMI
Poeschl
RJRT
SEITA/Imperial
Swedish Match NA
Swedish Match NE
Swisher
USSTMC/Altria

5. Samples

5.1 Selection

The products in Table 1 have been chosen to represent the brands of smokeless tobacco sold globally.

Table 1. Smokeless Tobacco Products Included in the Study

Product Type	Portion/Loose	Market	Brand	Manufacturer/ Supplier	Distributor
Snus	Loose	Europe	General	SMNE	SMNE
Snus	0.4g Pouch	Europe	Mocca Mint	Fielder & Lundgren (BAT)	SMNE
Moist Snuff	Loose	US	Copenhagen Snuff	UST	UST
Chewing Tobacco	Loose Leaf	US	Redman	SMNA	UST
Chewing Tobacco	Twist	Europe	Oliver Twist Tropical	House of Oliver Twist	SMNE
Hard Snuff/Pellet	Pellet	US	Camel Orbs	RJRT	UST
Nasal Snuff	Loose	Europe	Gletscher Prise	Poeschl Tabak	SMNE
Chewing - Gutkha Oral Flake	Flake	India	Gutkha Brand	ITC to purchase from market	SMNE
Moist Snuff	loose	US	Grizzly Wintergreen Long Cut	Conwood	UST

5.2 Supply

The manufacturers and suppliers will endeavour to provide the distributors with 5kg of the samples by June 26th 2009.

5.3 Preparation

With the exception of the Camel Orbs and Oliver Twist Tropical samples, the manufacturers and suppliers will despatch a homogenised single batch of product of suitable particle size (<4mm) to the distributors. The Oliver Twist Tropical samples will be homogenised by the distributors.

The two distributors will repackage 24 lots of ~200g of each sample into zip-lock plastic bags. These bags will then put into a further bag to reduce the risk of loss of volatiles from the samples and to discourage between sample contamination. The bags will then be frozen to ≤ -18ºC for a minimum of 24 hours prior to despatching to the participating laboratories.

5.4 Labelling

The distributors will label the samples A to I according the list provided by the study coordinator.

The following statement will be clearly displayed on the packaging:

“NOT FOR RESALE. SAMPLES ARE FOR TEST PURPOSES ONLY. SAMPLES ARE OF NO COMMERCIAL VALUE”

5.5 Transportation

NOTE: Under incorrect storage conditions there is a high risk of sample degradation which should be avoided.

The samples will be removed from the cryogenic storage facilities on the day of despatch. The samples will be distributed via express delivery by USTTC in the US and Swedish Match NE in Europe.
No additional cryogenic precautions will be put in place during the transportation process. The distributor will adhere to the laboratories’ despatch instructions as given in the document below.


June 09 ST sub group collab study Cl

The distributors will inform the laboratories of the actual despatch date so that the receiving laboratories can prepare for receipt of the samples.

5.6 Quantity

Each participating laboratory will receive ~200g of each product.

5.7 Receipt

The samples shall be **signed for immediately** upon receipt and be **stored in a freezer** until the analyses are performed.

5.8 Within Laboratory Sample Preparation

The samples shall be thawed at room temperature for at least 2 hours before use. After this initial thawing the samples shall be stored in a refrigerator in between use.

The Camel Orb samples should be ground using a mill to a fine powder and stored in airtight containers between use.

The Snus pouches should be cut into 2 halves directly into the extraction vessel. <u>Both Snus and paper are to be analysed.</u>

6. Analysis

6.1 Analytes

The following analyses should be performed by each participating laboratory *wherever possible*:-

Table 2. List of Analytes

Analysis	Unit
Water*	%
Moisture	%
Nicotine	%
pH	
Tobacco- specific nitrosamines (TSNA) including NNN, NNK, NAB and NAT	ppb

*This should be determined both before and after the analyses are performed.

Water
The analytical method shall be specific for water and not for oven volatiles.
It is recommended that CRM56 (Karl Fisher) or CRM57(GC) are used. These methods are available on the CORESTA web site.

Moisture
Each laboratory should use its own in-house validated analytical procedure.

Nicotine
The method of the analysis of nicotine should be specific for nicotine and not other alkaloids. It is recommended that CRM62 is followed.

pH

Each laboratory should follow as closely as possible the CDC method (Federal Register, Vol. 74, No. 4, 2009, pgs 712-718) as attached below:-


Federal Register -
CDC methods info Jar

For the purpose of this study an extraction stirring time of 30 minutes is recommended. If laboratories are in a position to take additional measurements at 5 and 15 minutes then this information can be added to the results spreadsheet. Laboratory environmental conditions are to be recorded.

TSNAs

The following method has been drafted by Swedish Match NE and should be adhered to as closely as possible by those laboratories using LCMSMS. If laboratories are unable to use LCMSMS then other technology may be used, e.g. GC-TEA.

Please also note that a TSNA calibration standard will be distributed by SMNE with the samples. Laboratories are requested to analyse this as a sample against their in-house calibrations and record the result on the TSNA worksheet.

6.2 Timing

The analyses shall be performed between **July27th and August 7th** and as close in time as possible.

6.3 Sampling

Moisture:
Each laboratory shall analyse 3 test portions (replicates), from each of the 9 samples under repeatability conditions **twice**, once before and once after the analyses are performed.

All other analytes:
Each laboratory shall analyse 3 test portions (replicates), from each of the 9 samples under repeatability conditions, i.e. within a short interval of time by the same operator, using the same calibration standard and without any intermediate recalibration of the apparatus, unless this is an integral part of performing the measurement.

It is of importance that the analyses will be performed as close as possible in time after the samples have been thawed. Nicotine, as the most volatile analyte shall, where possible, be analysed first.

7. Data Reporting

A description of the analytical procedure used to analyse the samples, including sample sizes, extraction techniques, detection limits, quantification limits and other relevant information shall be entered in the spreadsheet and sent together with the collected analytical data.

The individual test results shall be entered into the spreadsheet that is be provided. (2009 CORESTA ST Collaborative Study Data reporting Workbook.XLS)

All test results should be reported as is. (With no correction for moisture content).

Moisture:
Two sets of three test results will be reported for each of the 9 samples (performed before and after the analyses are performed).

All other analytes:
Three individual test results will be reported for each of the 9 samples.

The results shall not be rounded and, ideally, reported to at least one more digit than specified in the standard method used by the laboratory for the test**. (The number of significant figures are specified in the relevant sheet of the results workbook)**

As well as the analytical results the following information shall be reported:-

Comments from the operators on any deviation from the documented analytical procedure should be reported in the comments column of the spreadsheet.

Information regarding any irregularities or disturbances during the measurement, including change of operator, together with a statement as to which measurements were performed by which operator, and the reasons for any missing results.

The date when the samples were received.

The date when the samples were measured.

Any other relevant information.

The spreadsheet with the results of the analysis and the comments made shall be sent by e-mail to Linda Drake, Study Coordinator, by August 14th.

8. Statistical analysis of the data

Statistical analysis of data will be carried out by Alexander Hauleithner, JTI Austria, as per ISO 5725 (including Z scores).

9. Presentation of the results from the Collaborative study

The draft report of the Collaborative study will be circulated in September.

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Appendix 2

B. *New Business*

- Auditors' Report on FCA FY 2008/ 2007 Financial Statements
- Registration of Loan Originators Under the Secure and Fair Enforcement for Mortgage Licensing Act of 2008

C. *Reports*

- OE Quarterly Report

Closed Session *

- Update on OE Oversight Activities

Dated: January 5, 2009.

Roland E. Smith,

Secretary, Farm Credit Administration Board.

[FR Doc. E9–121 Filed 1–5–09; 4:15 pm]

BILLING CODE 6705–01–P

FEDERAL ELECTION COMMISSION

Sunshine Act Notices

AGENCY: Federal Election Commission.

DATE AND TIME: Thursday, January 8, 2009, at 10 a.m.

PLACE: 999 E Street, NW., Washington, DC (Ninth Floor).

STATUS: This meeting will be open to the public.

ITEMS TO BE DISCUSSED:

Correction and Approval of Minutes.

Management and Administrative Matters.

Individuals who plan to attend and require special assistance, such as sign language interpretation or other reasonable accommodations, should contact Mary Dove, Commission Secretary, at (202) 694–1040, at least 72 hours prior to the hearing date.

DATE AND TIME: Friday, January 9, 2009, at 10 a.m.

PLACE: 999 E Street, NW., Washington, DC.

STATUS: This meeting will be closed to the public.

ITEMS TO BE DISCUSSED:

Compliance matters pursuant to 2 U.S.C. 437g.

Audits conducted pursuant to 2 U.S.C. 437g, § 438(b), and Title 26, U.S.C.

Matters concerning participation in civil actions or proceedings or arbitration.

Internal personnel rules and procedures or matters affecting a particular employee.

* Session Closed—Exempt pursuant to 5 U.S.C. 552b(c)(8) and (9).

PERSON TO CONTACT FOR INFORMATION: Robert Biersack, Press Officer, Telephone: (202) 694–1220.

Darlene Harris,

Deputy Secretary of the Commission.

[FR Doc. E8–31465 Filed 1–6–09; 8:45 am]

BILLING CODE 6715–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Centers for Disease Control and Prevention

Notice Regarding Revisions to the Laboratory Protocol To Measure the Quantity of Nicotine Contained in Smokeless Tobacco Products Manufactured, Imported, or Packaged in the United States

AGENCY: Centers for Disease Control and Prevention (CDC), Department of Health and Human Services.

ACTION: Notice and Summary of Public Comments.

SUMMARY: This notice amends the uniform protocol for the analysis of nicotine, total moisture, and pH in smokeless tobacco products (''Protocol''). The Protocol, originally published in the **Federal Register** in 1999 (64 FR 14086) and revised in the **Federal Register** on March 14, 2008 (73 FR 13903), implements the requirement of the Comprehensive Smokeless Tobacco Health Education Act (CSTHEA) of 1986 (15 U.S.C. 4401 *et seq.,* Pub. L. 99–252) that each person manufacturing, packaging, or importing smokeless tobacco products shall annually provide the Secretary of Health and Human Services (HHS) with a specification of the quantity of nicotine contained in each smokeless tobacco product. CDC re-published the notice in the **Federal Register** on June 23, 2008 (73 FR 35395) concerning the revision of the Protocol (1) To make a technical change to correct the date when the first report of information under the revised Protocol is due and (2) to solicit public comments concerning a change in the Protocol that increased the volume of water in the pH determination from 10 mL to 20 mL, and (3) to solicit public comments concerning the addition of the following commercial smokeless tobacco product categories: dry snuff portion packs, snus, snus portion packs, and pellet or compressed. This Notice also includes a summary of public comments and CDC's response to them.

The Protocol as published in the **Federal Register** on March 14, 2008 (73 FR 13903), remains in effect with the technical correction to the date as described in the **Federal Register** notice published on June 23, 2008 (73 FR 35395).

DATES: First report of information due June 30, 2009, with subsequent submissions due by March 31 of each year.

FOR FURTHER INFORMATION, CONTACT: Matthew McKenna, M.D., Director, Office on Smoking and Health, Centers for Disease Control and Prevention, *Telephone:* (770) 488–5701.

SUPPLEMENTARY INFORMATION: Since the implementation of the Protocol in 1999, several smokeless tobacco product categories have entered the U.S. smokeless tobacco market including snus, low moisture snuff sold in portion pouches, and smokeless tobacco sold in a compressed, pellet form. Some of the new smokeless tobacco product categories differ physically from previous smokeless tobacco categories, prompting a revision to the Protocol to reflect the current state of the marketplace.

Through its review of the Protocol, CDC also determined that an increase in volume of deionized, distilled water would facilitate measurements of pH values. After evaluating information that was brought to the attention of CDC regarding low moisture smokeless tobacco products packaged in portion pouches, CDC conducted an independent comparison of pH measurements in a wide variety of low and high moisture smokeless tobacco products. The results of the comparison indicated an acceptable (less than 2%) level of change in pH values when measurements were taken with 20 mL deionized, distilled water compared to the volume of deionized, distilled water specified in the previous Protocol. Increasing the volume of water in the mixture ensured that the matrix was sufficiently fluid to facilitate ease of measure. Thus, it is anticipated that the change in the volume of liquid for pH determination will facilitate the ease of measure of smokeless tobacco pH for all currently marketed smokeless tobacco categories (i.e., plug, twist, moist snuff, dry snuff, snus, loose leaf, chew, moist snuff in portion pouches, smokeless tobacco compressed into a pellet, and dry snuff in portion pouches).

Summary of Public Comments and CDC's Response: On June 23, 2008, a notice (73 FR 35395) was published reflecting the above discussed revisions to the Protocol and to solicit public comment on these specific changes. Six comments were received by the CDC, a majority of which suggested alternative approaches. A summary of the

comments received and CDC's response follows.

One commenter expressed a concern for the Federal funding and overall direction of the ''smokeless tobacco program.''

The issues raised in this comment were beyond the scope of the Protocol and solicitation of public comment.

One commenter, on behalf of several smokeless tobacco manufacturers, agreed with the proposed revision of Section IV(B) (see below for Protocol) of the Protocol to increase the volume of deionized, distilled water to be used in pH measurements from 10mL to 20mL.

One commenter, on behalf of several smokeless tobacco manufacturers, suggested that ''some flexibility be incorporated into Section IV(B) of the Protocol by providing that, as long as a minimum of 20 mL of liquid and 2 grams of sample are utilized, then larger amounts of liquid and sample may be utilized provided they are in a 10 to 1 ratio.''

CDC appreciated the suggestion that there be flexibility in adjusting the quantity of liquid and sample so long as the ratio of liquid to sample is 10 to 1. In evaluating this suggestion, CDC determined that adopting such a change would deviate from principles of good scientific practice as it does not promote protocol consistency, contrary to the aims of a uniform analytical protocol. According to the Cooperative Centre for Scientific Research Relative to Tobacco (CORESTA), a central organization responsible for promoting tobacco-related cooperative research, ''[t]he development of standard methods is critically important in ensuring consistency and comparability of data reported by the association members and as part of regulatory reporting of data.'' [Further details on CORESTA's viewpoint and its objectives are available online at *http://www.coresta.org/Home_Page/PresentationCORESTA(Oct08).pdf.*] As the fundamental purpose of the Protocol is to implement a multi-site testing protocol, CDC concluded that the development of a uniform analytical protocol is paramount to ensuring sound scientific efforts.

One commenter, on behalf of several smokeless tobacco manufacturers, raised the following point regarding the categorization of smokeless tobacco products in Section I(F) of the Protocol:

> ''* * * many of these separate product 'categories' are essentially identical smokeless tobacco products for the purposes of sample preparation (e.g., Moist snuff and snus; Moist snuff portion packs and snus portion packs) * * * since a number of smokeless tobacco manufacturers have stated that they are developing new or 'innovative' smokeless tobacco products, an approach that creates a new 'category' and sample preparation instruction every time a smokeless tobacco product is introduced with a different name or description will result in a proliferation of smokeless tobacco product 'categories' and a need to constantly revise the Protocol to add new sample preparation instructions. Such revisions would trigger a notice and comment process under the Administrative Procedure Act.''

CDC made the determination to include the four newly listed categories after having reviewed the number and types of smokeless tobacco products that had entered the market since 1999. In this review, CDC concluded that several new products would benefit from a separate categorization to not only better aid manufacturers in distinguishing their products in this protocol, but also reflect the variety of products being sold to and recognized by consumers. This review also determined that in the years since the implementation of the Protocol in 1999, the quantities of new products introduced to market requiring separate categorization had been fairly limited; thus, CDC did not believe that constant revisions to the Protocol would be necessary. However, CDC will continue to monitor the introduction of new smokeless tobacco products and provide assistance to reporting entities on the application of the Protocol as needed.

One commenter, on behalf of several smokeless tobacco manufacturers, suggested an alternative approach that would ''eliminate, or at the least minimize, the need for new 'categories' and sample preparation instructions.''

This alternative proposal suggested that:

> ''The alternative approach would be to define the smokeless tobacco product categories based on physical characteristics relevant to sample preparation (essentially tobacco particle size and whether tobacco particles are in a pouch), rather than on a manufacturer's package label statement or description * * * ''

Three product categories were thus proposed.

If any products did not fall into the three categories, the proposal suggested that:

> ''* * * in the event that a smokeless tobacco manufacturer or importer believes that a newly marketed smokeless tobacco product does not fit within any of the above categories, then samples should be prepared in a manner compatible with the above sample preparation instructions and the manufacturer or importer should describe the sample preparation procedures used when making its submissions to CDC.''

After an evaluation of this alternative approach, CDC concluded that the current method of categorization is more appropriate for several reasons. First, the current method has been in place since 1999, with no noted difficulties associated with this product categorization. Second, CDC noted that other Federal agencies, such as the Federal Trade Commission (FTC) and United States Department of Agriculture (USDA), receive and review information on smokeless tobacco, not on the basis of physical size characteristics, but on these commonly accepted types of categories. Examples can be found in the FTC's ''Federal Trade Commission Smokeless Tobacco Report for the Years 2002–2005,'' available online at *http://www.ftc.gov/reports/tobacco/02-05smokeless0623105.pdf,* or in the USDA Economic Research Service's ''Tobacco Situation and Outlook Yearbook'', available online at *http://usda.mannlib.cornell.edu/usda/ers/TBS-yearbook//2000s/2007/TBS-yearbook-01-12-2007.pdf/.*

Furthermore, CDC viewed the existing categorization of products by traditional ''consumer-oriented'' descriptions as useful in easily identifying issues that concern the general consumer and the overall public's health. In contrast, adopting a method of categorization based solely on physical product characteristics would not be beneficial towards that goal.

Finally, during its review of this alternate approach, CDC noted that there are only three existing methods to prepare smokeless tobacco products for analysis in this protocol, despite the varied physical characteristics of currently marketed smokeless tobacco products.

One commenter, on behalf of several smokeless tobacco manufacturers, suggested that ''the reporting provision of the FRN be amended to provide the following: (i) The revised Protocol shall take effect January 1, 2009, and (ii) the first report of information pursuant to the revised Protocol is due March 31, 2010, with subsequent submissions due by March 31 of each year. This amendment would afford smokeless tobacco manufacturers a reasonable amount of time to prepare for the implementation of the revised Protocol, and would continue the current practice of manufacturers submitting a full year of data based on a consistent methodology.''

For the purposes of this comment, CDC took into consideration a **Federal Register** Notice published in March 2008 (73 FR 13903), which served as public notice about the changes in the Protocol. CDC regarded this duration of notice as sufficient for the first report of information to be due June 30, 2009,

with subsequent submissions due by March 31 of each year, as laid out in the June 23, 2008 **Federal Register** (73 FR 35395).

Collection of Information

This proposed amendment does not call for any new collection of information under the Paperwork Reduction Act of 1995 (44 U.S.C. 3501–3520).

Dated: December 29, 2008.

James D. Seligman,

Chief Information Officer, Centers for Disease Control and Prevention.

Revised Protocol for Analysis of Nicotine, Total Moisture, and pH in Smokeless Tobacco Products

I. Requirements [1] [2]

A. Reagents [3]

1. Sodium hydroxide (NaOH), 2N
2. Methyl t-butyl ether (MTBE)
3. (−)-Nicotine (Fluka 72290) >99% purity [4] [5]
4. Quinoline (Aldrich)
5. Standard pH buffers; 4.01, 7.00, and 10.00
6. Deionized distilled water

B. Glassware and Supplies

1. Volumetric flasks, class A
2. Culture tubes, 25 mm x 200 mm, with Teflon-lined screw caps
3. Pasteur pipettes
4. Repipettors (10 mL and 50 mL)
5. Linear shaker (configured to hold tubes in horizontal position) [6] [7]
6. Weighing dishes, aluminum
7. Teflon-coated magnetic stirring bars
8. Polypropylene containers, 50 mL

C. Instrumentation

1. Robot Coupe Model RSI 2V Scientific Batch Processor
2. Capillary gas chromatograph, Hewlett Packard, Model 6890, with split/splitless injector capability, flame ionization detector, and a capillary column (Hewlett Packard HP–5, Crosslinked 5% PH ME Siloxane, 30 m length x 0.32 mm ID, film thickness 0.25 or 0.52 μm)
3. Orion Model EA 940 pH meter equipped with Orion 8103 Ross combination pH electrode

D. Additional Equipment

Forced-air oven, Fisher Isotemp®, regulated to 99 ± 1.0°C. Suggested dimensions: 18 x 18 x 20inches.

E. Chromatographic Conditions [8] [9]

1. Detector temperature: 250°C
2. Injector temperature: 250°C
3. Flow rate at 100°C—1.7 mL/min; with split ratio of 40:1 [10]
4. Injection volume: 2 μl
5. Column conditions: 110–185°C at 10°C min^{-1}; 185–240°C at 6°C min^{-1}, hold at final temperature for 10 min.

F. Sample Preparation [11]

There are ten different categories of commercial smokeless tobacco products:
1. Dry snuff;
2. Moist (wet) snuff;
3. Moist (wet) snuff portion packs;
4. Plug;
5. Twist;
6. Loose leaf;
7. Dry snuff portion packs;
8. Snus;
9. Snus portion packs; and
10. Pellet or Compressed.

Because of their physical characteristics, some of the ten product categories must be ground (whole or in part) before nicotine, total moisture, and pH analyses can be conducted. The objective of grinding the samples is to obtain a homogeneous sample with particles measuring approximately 4 mm. Grinding to achieve this particle size should take no more than 3 minutes. To ensure proper grinding and an adequate amount of the ground sample for analysis, the minimum sample size of all commercial products to be ground should not be less than 100 grams.

To ensure precision of analyses for nicotine, total moisture, and pH, the samples that require grinding should be ground using a Robot Coupe Model RSI 2V Scientific Batch Processor or its equivalent. This is a variable speed (0 to 3000 RPM) processor. The variable speed motor is required to ensure proper grinding of the tobacco tissues (and in the case of pH determination, the portion pack). Elevated temperatures can result in moisture loss and an underestimated value for moisture content. Hence, care must be taken during grinding to avoid elevated temperatures. The bowl should be cleaned after each grinding to obtain accurate results. Freeze- or cryo-grinding is also an acceptable grinding method.

1. *Dry snuff:* Dry snuff samples do not need to be ground since the product is a powder. The sample must be thoroughly mixed before weighing for nicotine, total moisture, and pH analysis.

2. *Moist (wet) snuff:* Moist (wet) snuff samples do not need to be ground. The sample must be thoroughly mixed before weighing for nicotine, total moisture, and pH analysis.

3. *Moist (wet) snuff portion packs:* The tobacco contents of the moist (wet) snuff portion packs do not need to be ground for nicotine, total moisture, or pH analysis. The tobacco packaging material (the ''pouch'') should be separated from the tobacco and ground to obtain particles measuring approximately 4 mm for pH analysis. The tobacco of the moist (wet) snuff portion pack and the ground pouch are combined and thoroughly mixed before pH analysis.

4. *Plug tobacco:* Break or cut apart plugs and add in portions to grinder at 2000 RPM. Reduce RPM or stop grinding if sample bowl becomes warm. Pulse the Robot Coupe, when needed, to complete grinding. Grind samples until approximately 4 mm in size. The total grinding time should be no more than 3 minutes.

5. *Twist tobacco:* Separate twists, add to grinder and grind at 2000 RPM. Reduce RPM or stop grinding if sample bowl becomes warm. Continue grinding until sample particles are approximately 4 mm in size. The total time for grinding should be no more than 3 minutes.

6. *Loose leaf:* Grind in the same manner as described in 4 and 5 to obtain product with particle size of approximately 4 mm.

7. *Dry snuff portion packs:* The tobacco contents of the dry snuff portion packs do not need to be ground for nicotine, total moisture, or pH analysis. The tobacco packaging material (the ''pouch'') should be separated from the tobacco and ground to obtain particles measuring approximately 4 mm for pH analysis. The tobacco of the dry snuff portion pack and the ground pouch are combined and thoroughly mixed before pH analysis.

8. *Snus:* Snus samples do not need to be ground since the product is a powder. The sample must be thoroughly mixed before weighing for nicotine, total moisture, and pH analysis.

9. *Snus portion packs:* The tobacco contents of the snus portion packs do not need to be ground for nicotine, total moisture, or pH analysis. The tobacco packaging material (the ''pouch'') should be separated from the tobacco and ground to obtain particles measuring approximately 4 mm for pH analysis. The tobacco of the snus portion pack and the ground pouch are combined and thoroughly mixed before pH analysis.

10. *Pellet or compressed:* Break apart compressed tobacco pellets and add in portions to grinder at 2000 RPM. Reduce RPM or stop grinding if sample bowl becomes warm. Pulse the Robot Coupe, when needed, to complete grinding. Grind samples until approximately 4 mm in size. The total grinding time should be no more than 3 minutes.

II. Nicotine Analysis [12]

A. Calibration Standards

1. Internal Standard (IS)

Weigh 10.00 grams of quinoline, transfer to a 250 mL volumetric flask and dilute to volume with MTBE. This solution will be used for calibration of the instrument for the nicotine calibration curve (II.A.2), for the standards addition assay (II.B), and for preparation of the extracting solution (II.D).

2. Nicotine Calibration Curve

a. Weigh 1.0000 gram of nicotine into a clean, dry 100 mL volumetric flask and dilute to volume with MTBE. This gives a nicotine concentration of 10 mg/mL for the stock solution.

b. Accurately pipette 0.5 mL of IS from stock solution (II.A.1) to five clean, dry 50 mL volumetric flasks. To prepare a nicotine standard corresponding to a concentration of 0.8 mg/mL, pipette exactly 4.0 mL of the nicotine standard (II.A.2.a) to a 50 mL volumetric flask containing the internal standard and dilute to volume with MTBE. To obtain nicotine concentrations equivalent to 0.6, 0.4, 0.2, and 0.1 mg/mL, pipette precisely 3.0, 2.0, 1.0, and 0.5 mL, respectively, of the nicotine standard into the four remaining flasks and dilute to volume with MTBE.

c. Transfer aliquots of the five standards to auto sampler vials and determine the detector response for each standard using gas chromatographic conditions described in I.E.

d. Calculate least squares line for linear equation from these standards by obtaining the ratio of $Area_{nicotine}$/ $Area_{IS}$. This ratio will be the Y value and the concentration of nicotine will be the X value for determining the linear equation of the line (Equation 1):

Equation 1:

$$Y = a + bX;$$

Where:
X = Concentration of nicotine in mg
Y = $Area_{nicotine}$/ $Area_{IS}$
a = intercept on the ordinate (y axis)
b = slope of the curve

The final result will be reported in the following units:

Concentration of nicotine = mg of nicotine/gram of tobacco sample.

e. Determine the recovery of nicotine by pipetting 10 mL of the 0.4 mg/mL nicotine standard to a screw capped tube containing 1.0 mL of 2 N NaOH. Cap the tube. Shake the contents vigorously and allow the phases to separate. Transfer an aliquot of the organic phase to an injection vial and inject. Calculate the concentration of nicotine using the equation of the line in II.A.2.d above. This should be repeated two more times to obtain an average of the three values. The recovery of nicotine can be obtained by using the following equation:

Equation 2:

$$Recovery = Nicotine_{calculated}/Nicotine_{actual}$$

B. Standards Addition Assay

Prior to analyzing a smokeless tobacco product for nicotine content, the testing facility must validate the system to verify that matrix bias is not occurring during nicotine extraction. This is done by analyzing the nicotine calibration standards in the same vegetable matrix as the smokeless tobacco. The first time each smokeless tobacco product is tested and whenever a change is made to the product formulation (including a change to the tobacco blend or cultivar), the Standards Addition Assay will be performed, and documentation of its performance and of the nicotine concentrations selected for the standard curve (II.B.2) will be submitted to the Centers for Disease Control and Prevention.

1. Using an analytical balance, accurately weigh 1.000 ± 0.020 gram of the homogeneous, prepared tobacco sample into a culture tube. Repeat this five times for a total of 6 culture tubes containing the smokeless tobacco product. Record the weight of each sample.

2. Prepare a five-point standard curve for the Standards Addition Assay. The standard curve must consist of nicotine concentrations that encompass the range of values expected from adding known concentrations of the nicotine standard (II.A.2.a) to a measured quantity of the smokeless tobacco product (1.000 ± 0.020 gram, described in II.B.1). The sixth culture tube is not supplemented with nicotine and serves as an analytical blank. Allow the samples to equilibrate for 10 minutes.

3. Pipette 5 mL of 2 N NaOH into each tube. Cap each tube. Swirl to wet sample and allow to stand 15 minutes.[13]

4. Pipette 50 mL of extraction solution (II.D.1) into each tube. Cap each tube and tighten.[14]

5. Place tubes in rack(s), place racks in linear shaker in horizontal position and shake for two hours.

6. Remove rack(s) from shaker and place in vertical position to allow the phases to separate.

7. Allow the solvent and nicotine supplemented samples and the blank to separate (maximum 2 hours).

8. Transfer aliquots of the five standards and the blank from the extraction tubes to sample vials and determine the detector response for each using gas chromatographic conditions described in I.E.

9. Subtract the $Area_{nicotine}/Area_{IS}$ of the blank from the $Area_{nicotine}/Area_{IS}$ of each of the standards.

10. Calculate least squares line for linear equation from the corrected standards as described above (Equation 1) in II.A.2.d. The final corrected result will be reported in the following units: Concentration of nicotine = mg of nicotine/gram of tobacco sample.

11. Determine the recovery of nicotine by pipetting 10 mL of the 0.4 mg/mL nicotine standard to a screw capped tube containing 1.0 mL of 2 N NaOH and 10 mL of extraction solution (II.D.1). Cap the tube and tighten. Shake the contents vigorously and allow the phases to separate. Transfer an aliquot of the organic phase to an injection vial and inject. Calculate the concentration of nicotine using the equation of the line above in II.A.2.d. This should be repeated two more times to obtain an average of the three values. The recovery of nicotine can be obtained by using Equation 2: Recovery = $Nicotine_{calculated}/Nicotine_{actual}$.

12. Compare the results of steps II.A.2 and II.B. If they differ by a factor of 10% or more, the recovery of nicotine from the aqueous matrix is not equivalent to recovery from the vegetable matrix of the smokeless tobacco product. In this instance, the nicotine concentration of the smokeless tobacco product must be determined from a nicotine calibration curve prepared from nicotine standards in a vegetable-based matrix.

C. Quality Control Pools

At least two quality control pools at the high and low ends of the expected nicotine values are recommended to be included in each analytical run. The pools should be analyzed in duplicate in every run. The quality control pools should be available in sufficient quantity to last for all analyses of a product.

D. Sample Extraction Procedure [12]

1. Extraction solution is prepared by pipetting 10 mL of the IS from the stock solution (II.A.1) to a 1000 mL volumetric flask and diluting to volume with MTBE.

2. Using an analytical balance, accurately weigh 1.000 ± 0.020 gram of prepared tobacco sample into culture tube and record weight.[15] Sample each smokeless tobacco brand name according to the provided testing frequency schedule.[19] The number of

products sampled should reflect an acceptable level of precision.[16] The test material is to be representative of the product that is sold to the public and therefore should consist of sealed, packaged samples of finished product that is ready for commercial distribution. Samples are to be analyzed in duplicate.

3. Pipette 5 mL of 2 N NaOH into the tube. Cap the tube. Swirl to wet sample and allow to stand 15 minutes.[13]

4. Pipette 50 mL of extraction solution into tube, cap tube and tighten.[14]

5. Place tubes in rack(s), place racks in linear shaker in horizontal position and shake for two hours.

6. Remove rack(s) from shaker and place in vertical position to allow the phases to separate.

7. Allow the solvent and sample to separate (maximum 2 hours). Transfer an aliquot from the extraction tube to a sample vial and cap.

8. Analyze the extract using GC conditions as described above (I.E) and calculate the concentration of nicotine using the linear calibration equation. Correct percent nicotine values for both recovery and weight of sample by using Equation 3.[17]

Equation 3:[18]

$$\text{Nicotine (mg/g)} = \frac{\left(\text{Area}_{\text{nicotine}}/\text{Area}_{\text{IS}}\right) - a}{b \times \text{Sample Wt} \times \text{Recovery}}$$

9. Report the final nicotine determination as mg of nicotine per gram of the tobacco product (mg nicotine/gram), to an accuracy level of two decimal places for each brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.). All data should include the mean value with a 95% confidence interval, the range of values, the number of samples tested, the number of lots per brand name, and the estimated precision of the mean. Information will be reported for each manufacturer and variety (including brand families and brand variations) and brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.).

III. Total Moisture Determination

A. This procedure is a modification of AOAC Method 966.02 (1990) and is referred to as ''Total Moisture Determination'' because it determines water and tobacco constituents that are volatile at temperatures of 99 ± 1.0°C.

B. Accurately weigh 5.00 grams of the sample (ground to pass ≤ 4 mm screen)[20] into a weighed moisture dish and place uncovered dish in oven.[21] Sample each smokeless tobacco brand name according to the provided testing frequency schedule.[19] The number of products sampled should reflect an acceptable level of precision.[16] The test material is to be representative of the product that is sold to the public and therefore should consist of sealed, packaged samples of finished product that is ready for commercial distribution. Samples are to be analyzed in duplicate.

C. Do not exceed 1 sample/10 sq in (650 sq cm) shelf space, and use only 1 shelf. Dry 3 hr at 99 ± 1.0°C. Remove from oven, cover, and cool in desiccator to room temperature (about 30 min). Reweigh and calculate percent moisture.

D. Report the final moisture determination as a percentage (%), to an accuracy level of one decimal place for each brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.). All data should include the mean value with a 95% confidence interval, the range of values, the number of samples tested, the number of lots per brand name, and the estimated precision of the mean. Information will be reported for each manufacturer and variety (including brand families and brand variations) and brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.).

IV. pH Measurement [12 22]

A. Test samples as soon as possible after they are received. Sample each smokeless tobacco brand name according to the provided testing frequency schedule.[19] The number of products sampled should reflect an acceptable level of precision.[16] The test material is to be representative of the product that is sold to the public and therefore should consist of sealed, packaged samples of finished product that is ready for commercial distribution. Samples are to be analyzed in duplicate.

B. Accurately weigh 2.00 grams of the sample. Place in a 50 mL polypropylene container with 20 mL deionized distilled water.

C. Place Teflon-coated magnetic stirring bar in container and stir mixture continuously throughout testing.

D. Measure pH of sample after a two-point calibration of the pH meter to an accuracy of two decimal places using standard pH buffers (4.01 and 7.00 or 7.00 and 10.00) that will encompass the expected pH value of the smokeless tobacco product.

E. The first time pH values are determined for a smokeless tobacco product, measure the pH of the smokeless tobacco product at 5, 15, and 30 minutes. If there is no systematic variation in pH values with time, all subsequent pH determinations are made at 5 minutes. If there is systematic variation in pH values, continue to measure the pH of the smokeless tobacco product until the pH value is stable and does not vary more than 10% over 15 minutes. Report the final pH value.

F. Report the final pH determination to an accuracy level of two decimal places for each brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.). All data should include the mean value with a 95% confidence interval, the range of values, the number of samples tested, the number of lots per brand name, and the estimated precision of the mean. Information will be reported for each manufacturer and variety (including brand families and brand variations) and brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.).

G. Estimate the un-ionized (free) nicotine content with the Henderson-Hassel Balch equation (Equation 4), based on measured pH and nicotine content.

Equation 4:

$$pH = pKa + \log\frac{[B]}{[BH^+]}$$

$$B + H^+ \rightleftharpoons BH^+$$

$$\%\ \text{un-ionized (free) nicotine} = \frac{\frac{[B]}{[BH^+]}}{\frac{[B]}{[BH^+]}+1}\times 100$$

pKa = 8.02 (CRC Handbook of Chemistry and Physics, 1989-1990)
[B] = amount of un-ionized (free) nicotine
$[BH^+]$ = amount of ionized nicotine

H. Report the final estimated un-ionized (free) nicotine as a percentage (%) of the total nicotine content, to an accuracy level of two decimal places and as mg of un-ionized (free) nicotine per gram of the tobacco product (mg un-ionized (free) nicotine/gram), to an accuracy level of two decimal places for each brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.). All data should include the mean value with a 95% confidence interval, the range of values, the number of samples tested, the number of lots per brand name, and the estimated precision of the mean. Information will be reported for each manufacturer and variety (including brand families and brand variations) and brand name (e.g., Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.).

Sample calculation:

Mean total nicotine = 10.30 (mg/g)

Mean pH = 7.50

pKa = 8.02

$$pH = pKa + \log\frac{[B]}{[BH^+]}$$

$$7.50 = 8.02 + \log\frac{[\text{un-ionized (free) nicotine}]}{[\text{ionized nicotine}]}$$

$$-0.52 = \log\frac{[\text{un-ionized (free) nicotine}]}{[\text{ionized nicotine}]}$$

$$0.302 = \frac{[\text{un-ionized (free) nicotine}]}{[\text{ionized nicotine}]}$$

$$\%\ \text{un-ionized (free) nicotine} = \frac{\frac{[B]}{[BH^+]}}{\frac{[B]}{[BH^+]}+1}\times 100$$

$$\%\ \text{un-ionized (free) nicotine} = \frac{0.302}{0.302+1}\times 100$$

% un-ionized (free) nicotine = 23.20

$$\text{Total free nicotine (mg/g)} = \text{total nicotine} \times \frac{\%\ \text{un-ionized (free) nicotine}}{100}$$

$$\text{Total free nicotine (mg/g)} = 10.30 \times \frac{23.20}{100}$$

Total free nicotine (mg/g) = 2.39

V. Assay Criteria for Quality Assurance

A. Establishing Limits for Quality Control Parameters

All quality control parameters must be determined within the laboratory in which they are to be used. At least 10 within-laboratory runs must be performed to establish temporary confidence intervals for the quality control parameters. Permanent limits should be established after 20 runs and should be reestablished after each additional 20 runs.

B. Exclusion of Outliers from the Calibration Curve [18]

The coefficient of determination between $Area_{nicotine}/Area_{IS}$ and nicotine concentration should be equal to 0.99 or higher. Any calibration standard having an estimated concentration computed from the regression equation (Equation 1) which is different from its actual concentration by a factor of 10% can be excluded from the calibration curve. Up to two concentrations may be excluded, but caution should be used in eliminating values, since bias may be increased in the calibration curve. If an outlier value is eliminated, its duplicate value must also be discarded to avoid producing a new bias. All unknowns must fall within the calibration curve; therefore, duplicate values excluded at either end of the calibration curve will restrict the useful range of the assay.

C. Quality Control Pools and Run Rejection Rules

The mean estimated nicotine concentration in a pool should be compared with the established limits for that pool based on at least 20 consecutive runs. An analytical run should be accepted or rejected based upon the following set of rules adapted from Westgard *et al.* (1981).

1. When the mean of one QC pool exceeds the limit of $x \pm 3$ standard deviations (SD), then the run is rejected as out of control. Here, x and SD represent the overall mean and standard deviation of all estimated nicotine concentrations for a particular pool in the runs which were used to establish the control limits.

2. When the mean nicotine concentrations in two QC pools in the same run exceed the same direction, then the run must be rejected. The same direction is the condition in which both pools exceed either the x + 2 SD or the x -2 SD limits.

3. When the mean nicotine concentrations in one or two QC pools exceed their $x \pm 2$ SD limits in the same direction in two consecutive runs, then both runs must be rejected.

4. When the mean nicotine concentrations in two QC pools are different by more than a total of 4 SD, then the run must be rejected. This condition may occur, for example, when one QC pool is 2 SD greater than the mean, and another is 2 SD less than the mean.

Endnotes

The comments and notes listed below can be described as Good Laboratory Practice guidelines; they are described in detail in this protocol to ensure minimal interlaboratory variability in the determination of nicotine, total moisture, and pH in smokeless tobacco.

[1] This protocol assumes that the testing facility will implement and maintain a stringent Quality Assurance/Quality Control program to include, but not be limited to, regular interlaboratory comparisons, determination of the quality and purity of purchased products, and proper storage and handling of all reagents and samples.

[2] When a specific product or instrument is listed, it is the product or instrument that was used in the development of this method. Equivalent products or instruments may also be used. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

[3] All chemicals, solvents, and gases are to be of the highest purity.

[4] Companies must ensure that the purity of the nicotine base is certified by the vendor and that the chemical is properly stored. However, nicotine base oxidizes with storage, as reflected by the liquid turning brown. If oxidation has occurred, the nicotine base should be distilled prior to use in making a standard solution.

[5] A suggested method for the determination of nicotine purity is CORESTA Recommended Method No. 39.

[6] Horizontal shaking will allow more intimate contact of this three phase extraction. There is a minimal dead volume in the tube due to the large sample size and extraction volume. This necessitates horizontal shaking.

[7] If a linear shaker is not available, a wrist action shaker using 250 mL stoppered Erlenmeyer flasks can be substituted. Values for nicotine are equivalent to those obtained from the linear shaker.

[8] After installing a new column, condition the column by injecting a tobacco sample extract on the column, using the described column conditions. Injections should be repeated until areas of IS and nicotine are reproducible. This will require approximately four injections. Recondition column when instrument has been used infrequently and after replacing glass liner.

[9] Glass liner and septum should be replaced after every 100 injections.

[10] Most older instruments operate at constant pressure. To reduce confusion, it is suggested that the carrier gas flow through the column be measured at the initial column temperature.

[11] The testing facility must ensure that samples are obtained through the use of a survey design protocol for sampling ''at one point in time'' at the factory or warehouse. The survey design protocol must address short-, medium-, and long-term smokeless tobacco product variability (*e.g.*, variability over time and from container to container of the tobacco product) in a manner equivalent to that described for cigarette sampling in Annex C of ISO Protocol 8243. Information accompanying results for each sample should include, but not be limited to:

For each product—manufacturer and variety (including brand families and brand variations) and brand name (*e.g.*, Skoal Bandits, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.):

1. Product ''category,'' *e.g.*, loose leaf, plug, twist, dry snuff, moist (wet) snuff, etc.

2. Lot number.

3. Lot size.

4. Number of randomly sampled, sealed, packaged (so as to be representative of the product that is sold to the public) smokeless tobacco products selected (sampling fraction) for nicotine, moisture, and pH determination.

5. Documentation of method used for random sample selection.

6. '''Age'' of product when received by testing facility and storage conditions prior to analysis.

[12] Extraction of nicotine and pH determination must be performed with reagents and samples at a room temperature of 22–25°C. Room temperature should not vary more than 1°C during extraction of nicotine or pH determination.

[13] Use non-glass 10 mL repipette for transferring NaOH solution.

[14] Use 50 mL repipette for transferring MTBE.

[15] For dry snuff, use 0.500 ± 0.010 gram sample.

[16] The testing facility is referred to ISO Procedure 8243 for a discussion of sample size and the effect of variability on the precision of the mean of the sample (ISO 8243, 1991).

[17] When analyzing new smokeless tobacco products, extract product without IS to determine if any

components co-elute with the IS or impurities in the IS. This interference could artificially lower calculated values for nicotine.

[18] The calculated nicotine values for all samples must fall within the low and high nicotine values used for the calibration curve. If not, prepare a fresh nicotine standard solution and an appropriate series of standard nicotine dilutions. Determine the detector response for each standard using chromatographic conditions described in I.E.

[19] The testing frequency for each smokeless tobacco brand name (*e.g.*, Skoal Bandits Wintergreen, Skoal Long Cut Cherry, Skoal Long Cut Wintergreen, etc.) is based on the manufacturing duration (refer to table below). Each smokeless tobacco brand name will be sampled and tested for nicotine, total moisture, and pH no fewer than twice and no more than four times during a calendar year.

Manufacturing duration in weeks	Test frequency*
up to and including 4	2
up to and including 28	3
up to and including 52	4

*Use a statistical program to determine random sampling dates based on the total manufacturing duration during a calendar year. Sampling dates should fall on actual manufacturing days for the product when test material that is representative of the product that is sold to the public (consisting of sealed, packaged samples) is available. If a statistically determined sampling date falls on a day that does not meet this criterion, sample the product on the next date that does meet the criteria.

For smokeless tobacco brand names with episodic production during a calendar year, the total number of sampling dates is determined by the sum of the individual test frequencies, not to exceed four. For the purpose of the Protocol, episodic production is defined as manufacturing intervals separated by periods of 30 or more days when the smokeless tobacco brand name is not manufactured.

Example 1: Within a single calendar year a smokeless tobacco brand name is manufactured from January 1 to March 31 and from September 1 to December 15. The testing frequency for the first manufacturing interval is 3 and for the second manufacturing interval is 3. The Protocol allows that each smokeless tobacco brand name be tested for nicotine, total moisture, and pH no more than four times during a calendar year. Therefore, 4 random sampling dates, as described in the footnote to the above table, are determined for the smokeless tobacco brand name. The values for nicotine, moisture, and pH determinations, and unionized (free) nicotine calculations and the mean of the 4 data points for that smokeless tobacco brand name are reported.

Example 2: Within a single calendar year a smokeless tobacco brand name is manufactured from April 5 to May 3 and from September 1 to December 15. The testing frequency for the first manufacturing interval is 2 and for the second manufacturing interval is 3. The values for nicotine, moisture, and pH determinations, and unionized (free) nicotine calculations and the mean of the 4 data points for that smokeless tobacco brand name are reported.

Example 3: Within a single calendar year a smokeless tobacco brand name is manufactured from January 1 to January 15 and from September 1 to September 22. The testing frequency for the first manufacturing interval is 2 and for the second manufacturing interval is 2. Four random sampling dates are selected to fall within the 6 weeks of manufacturing for the smokeless tobacco brand name. The values for nicotine, moisture, and pH determinations, and unionized (free) nicotine calculations and the mean of the 4 data points for that smokeless tobacco brand name are reported.

[20] The method is a modification of AOAC Method 966.02 (1990) in that the ground tobacco passes through a 4 mm screen rather than a 1 mm screen.

[21] When drying samples, do not dry different products (e.g., moist (wet) snuff, dry snuff, loose leaf) in the oven at the same time since this will produce errors in the moisture determinations.

[22] The method is a modification of a method published by Henningfield *et al.* (1995).

References

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Center for Scientific Review; Notice of Closed Meetings

Pursuant to section 10(d) of the Federal Advisory Committee Act, as amended (5 U.S.C. Appendix 2), notice is hereby given of the following meetings.

The meetings will be closed to the public in accordance with the provisions set forth in sections 552b(c)(4) and 552b(c)(6), Title 5 U.S.C., as amended. The grant applications and the discussions could disclose confidential trade secrets or commercial property such as patentable material, and personal information concerning individuals associated with the grant applications, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

Name of Committee: Center for Scientific Review, Special Emphasis Panel, Member Conflict: Auditory Neuroscience.

Date: January 22–23, 2009.

Time: 6 a.m. to 5 p.m.

Agenda: To review and evaluate grant applications.

Place: National Institutes of Health, 6701 Rockledge Drive, Bethesda, MD 20892. (Virtual Meeting)

Contact Person: John Bishop, PhD, Scientific Review Officer, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5180, MSC 7844, Bethesda, MD 20892, (301) 435–1250, *bishopj@csr.nih.gov.*

Name of Committee: Center for Scientific Review, Special Emphasis Panel, Epidemiology and Genetics of Aging and Neurodegenerative Diseases.

Date: January 23, 2009.

Time: 12 p.m. to 3 p.m.

Agenda: To review and evaluate grant applications.

Place: National Institutes of Health, 6701 Rockledge Drive, Bethesda, MD 20892. (Telephone Conference Call)

Contact Person: Fungai F. Chanetsa, PhD, Scientific Review Officer, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 3135, MSC 7770, Bethesda, MD 20892, 301–435–1262, *chanetsaf@csr.nih.gov.*

Name of Committee: Center for Scientific Review, Special Emphasis Panel, Member Conflicts: Alcohol and Toxicology.

CSTS Collaborative Study 2009
Technical Report

Appendix 3

Appendix 2

Collaborative study for Quantification of TSNA by LC-MS/MS in Smokeless Tobacco Products

Scope of Application

The method to be used in this study is applicable for the quantification of four tobacco specific N-nitrosamines (TSNAs) in Smokeless Tobacco Products. The TSNAs analyzed with this method are N-nitrosonornicotine (NNN), 4-(N-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N-nitrosoanatabine (NAT), and N-nitrosoanabasine (NAB).

Principle

The TSNAs are extracted with an aqueous buffer and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results are reported in units of nanograms per gram product as is, wet weight.

Safety Precautions

TSNAs are category 2 carcinogens, toxic and may impair fertility and as such should be treated with extreme care, and should always be handled in a fume cupboard. Lab coat, glasses and chemical resistant gloves should always be worn.

Equipment

Laboratories have the possibility to either use the UPLC column or the HPLC column specified below. The reason for the use of the UPLC column is to increase the sensitivity in order to be able to detect the low levels of TSNA in some smokeless tobacco products. In addition the run time with UPLC is only about half of the HPLC run time.

Usual laboratory equipment and in particular, the following:

Conical flask: 100 mL with stopper.
Calibrated auto pipettes: 100-1000 µL.
Calibrated dispensette for 30 mL buffer.
Vials: Whatman Mini-UniPrep ^{TM} PTFE (PolyTetraFluoroEthylene) filter 0.45 µm,
(or 2 mL amber vials, syringe and 0.45 µm PVDF- or PTFE syringe filter).
Laboratory balance, capable of weighing to the nearest 0.0001 g
Orbital shaker.
pH-meter.

UPLC
Participating laboratories with possibility to use UPLC or similar system (UHPLC) should use that.
UPLC column: Waters Acquity UPLC BEH C18 column, 2.1mm x 100mm,
1.7µm particle size.
Frit: Waters Assay Frit, 0.2 µm, 2.1 mm.

HPLC
Laboratories without the possibility to use UPLC should use HPLC:
HPLC column: Waters Xterra MS C18 column, 2.1 x 50 mm, 2.5 µm particle size
Guard column: Waters Xterra MS C18 guard column, 2.1 x 10 mm, 3.5 µm particle size

Triple Quadrupole Mass Spectrometer: Participating laboratories will use their in-house validated system and parameters. For smokeless tobacco products a sensitive instrument is required since some of the smokeless products have low levels of TSNA.

Reagents and Supplies

During the analysis, use only reagents of recognized analytical grade and only Milli-Q grade water or water of grade 1 according to EN ISO 3696. Solvents should be of HPLC-grade or better.

Milli-Q water	(HPLC-grade)
Acetonitrile	(HPLC-grade).
Ammoniumacetate	(p.a. grade).
Methanol	(HPLC-grade).
Acetic acid	(98-100 %, p.a. grade).
Formic acid	(> 90 %, p.a. grade).
NAB	(≥ 98 %)
NAT	(≥ 98%)
NNK	(≥ 98 %)
NNN	(≥ 98 %)
NAB-d4	(≥ 98 %, Isotopic purity ≥ 99%)
NAT-d4	(≥ 98 %, Isotopic purity ≥ 99%)
NNN-d4	(≥ 98 %, Isotopic purity ≥ 99%)
NNK-d4	(≥ 98 %, Isotopic purity ≥ 99%)

Sample Handling and Storage

See the collaborative study main protocol for instructions.

Analytical Procedure – Solution Preparation

100 mM ammonium acetate solution (To be used for Extraction Solution and for Mobile phase preparation)

100 mM ammonium acetate in Milli-Q Water: Weigh 15.40 grams of ammonium acetate and transfer the ammonium acetate into a 2000-mL volumetric flask. Add ~1000 mL of Milli-Q Water and mix well, dilute to volume with Milli-Q Water and mix well. Store at room temperature.

Mobile Phases for the UPLC system

Mobile phase A: 10 mM ammonium acetate buffer, pH 4.7 (± 0.05)

Mix 100 mL of 100 mM ammonium acetate solution with 900 mL Milli-Q water, adjust the pH to 4.7 (± 0.05) with acetic acid.

Mobile phase B: 0.1 % (v/v) formic acid in acetonitrile

Pipette 1.0 mL of formic acid using a calibrated auto pipette (or a class A pipette), into a 1000 mL volumetric flask containing about 600 mL acetonitrile, dilute to volume with acetonitrile and mix well. Store at room temperature.

Mobile Phases for the HPLC system

Mobile phase A: Mill-Q water

Mobile phase B: 0.1 % (v/v) acetic acid in methanol

Pipette 1.0 mL of acetic acid using a calibrated auto pipette (or a class A pipette), into a 1000 mL volumetric flask containing about 600 mL methanol, dilute to volume with methanol and mix well. Store at room temperature.

Standard Diluent

30/70 Acetonitrile/Milli-Q Water, Combine 300 mL of acetonitrile and 700 mL of Milli-Q Water into a 1 L bottle. Mix well.

Preparation of Standards

Each laboratory will prepare its own standards. A TSNA standard control solution will be distributed to all participants to control the calibration standards. All Standards should be prepared in amber, or light protected glassware. Store standard solutions described below in a refrigerator at ~ 4^{o}C. The solutions are brought to room temperature before use.

Intermediate TSNA Standard Solution 1:

Prepare 100 mL Intermediate Standard solution containing 40, 40, 40 and 10 µg/mL of NNN, NNK, NAT and NAB in acetonitrile, and mix well.

Intermediate TSNA Standard Solution 2:

Prepare 100 mL standard solution containing 400, 400, 400 and 100 ng/mL for NNN, NNK, NAT and NAB respectively in 30/70 acetonitrile/ Milli-Q Water by:

Add 1000 µL of Intermediate TSNA Standard Solution 1 in a 100-mL volumetric flask, use a class A pipette (or calibrated auto pipettes), dilute to volume with 30/70 acetonitrile/Mill-Q Water, and mix well.

Intermediate TSNA Standard Solution 2b (for preparation of QC):

Prepare 100 mL standard solution containing 400, 400, 400 and 100 ng/mL for NNN, NNK, NAT and NAB respectively in 30/70 acetonitrile/ Milli-Q Water by:

Add 1000 µL of Intermediate TSNA Standard Solution 1 in a 100-mL volumetric flask, use a class A pipette (or calibrated auto pipettes), dilute to volume with 30/70 acetonitrile/Mill-Q Water, and mix well.

Intermediate Internal Standard Solution

Prepare a 100 mL Intermediate Internal standard solution containing 10µg/mL of NNN-d4, NNK-d4, NAT-d4 and NAB-d4 in acetonitrile and mix well.

Internal Standard Spiking Solution

Prepare a 100 mL Internal Standard Spiking Solution containing 2000 ng/mL of NNN-d4, NNK-d4, NAT-d4 and NAB-d4 in acetonitrile by:

Add 20 mL of Intermediate Internal Standard Solution (10µg/mL) in a 100-mL volumetric flask, use a class A pipette, dilute to volume with acetonitrile, and mix well.

TSNA Calibration Standards

The TSNA Calibration Standards are prepared in eight separate, 100-mL volumetric flasks each containing 10 mL of 100 mM ammonium acetate solution. Add 1.0 mL of the Internal Standard Spiking Solution (2000 ng/mL) to each of the eight volumetric flasks using a class A pipette (or a calibrated pipette). Next, the appropriate volume of Intermediate TSNA Standard Solution 2, given in table 1 below, is added. Add then the volume of acetonitrile, given in table 1 below. Finally each of the seven flasks is diluted to volume with 100 mM ammonium acetate, and mix well.

Table 1. Concentration and preparation of TSNA Calibration Standards

Cal. Std.	Volume of TSNA Intermediate std. Nr. 2 (mL)	Volume of Standard Spiking Solution 2000 ng/mL (mL)	Volume acetonitrile (mL)	Conc. NNN (ng/mL)	Conc. NNK (ng/mL)	Conc. NAT (ng/mL)	Conc. NAB (ng/mL)
Cal 1	0.125	1.00	22	0.5	0.5	0.5	0.125
Cal 2	0.250	1.00	22	1.0	1.0	1.0	0.250
Cal 3	0.50	1.00	22	2.0	2.0	2.0	0.50
Cal 4	1.00	1.00	22	4.0	4.0	4.0	1.00
Cal 5	2.00	1.00	22	8.0	8.0	8.0	2.00
Cal 6	5.00	1.00	21	20	20	20	5.00
Cal 7	10.0	1.00	19	40	40	40	10.0
Cal 8	25.0	1.00	15	100	100	100	25.0

TSNA Standard Control (TSC)

The purpose of the TSC is to examine to what extent any possible differences between laboratories are caused by difference in calibration standards. The TSC are prepared at each laboratory from a TSNA standard delivered to all participating laboratories together with the Smokeless Tobacco Product samples.

The TSC is prepared at each laboratory in an identical manner as the TSNA Calibration Standards. The TSC is prepared in 100-mL volumetric flasks containing 10 mL of 100 mM ammonium acetate solution and 1 mL of the Internal Standard Spiking Solution (2000

ng/mL). Add **5.00** mL of the enclosed TSNA Standard Control solution and 21 mL acetonitrile, and dilute to volume with 100 mM ammonium acetate. The results of the TSC should be reported in the result report.

Quality Control Standard (QC)

The QC Standards are prepared in an identical manner to the TSNA Calibration Standards, but from Intermediate standard 2**b**. Two QC standards of different concentrations are to be prepared to check the calibrating curve. The QCs are prepared in 100-mL volumetric flasks containing 10 mL of 100 mM ammonium acetate solution and 1.00 mL of the Internal Standard Spiking Solution (2000 ng/mL). Add the appropriate volumes of the TSNA Standard Solution 2**b** and acetonitrile, given in the table 2 below, and dilute to volume with 100 mM ammonium acetate.

Table 2. Concentration and preparation of TSNA Quality Control Standards

QC	Volume of TSNA Intermediate std. Nr. **2b** (mL)	Volume of Internal Standard Spiking Solution 2000 ng/mL (mL)	Volume acetonitrile (mL)	Conc. NNN (ng/mL)	Conc. NNK (ng/mL)	Conc. NAT (ng/mL)	Conc. NAB (ng/mL)
QC 1	3.00	1.00	22	12	12	12	3
QC 2	15.0	1.00	18	60	60	60	15

If the laboratory usually analyzes other QCs (e.g. control samples, standard reference materials or matrix spikes), then these QCs should be analyzed in addition to the samples analyzed in this study to ensure the quality of the analysis.

Analytical Procedure – Within Laboratory Sample Preparation

For instructions see study main protocol.
The samples shall be thawed at room temperature for 2 hours before use. If not all samples are analyzed during the same day, the samples shall be kept in a refrigerator (not freezer) after the initial thawing.

Snus pouches are cut into 2 halves directly into the extraction vessel. **Both** Snus and paper are analysed together.

The Camel Orbs samples should be ground using a mill to a fine powder and stored in airtight containers.

Analytical Procedure - Sample Extraction

1. Weigh out ~1.0 ± 0.1 grams (note the exact weight with 4 decimals) of sample and transfer into a 100 mL conical flask
2. Add 0.300 mL of the 2000 ng/mL Internal Standard Spiking Solution (using a calibrated Eppendorf pipettor (or equivalent)).
3. Add 30 mL of 100 mM ammonium acetate and cap the conical flask.
4. Shake the sample(s) on an orbital shaker for 40 minutes at 130 rpm.
5. Filter each sample using Whatman Mini-UniPrep ™ PTFE (PolyTetraFluoroEthylene) filter 0.2 µm, or a syringe with 0.45 µm PVDF- or PTFE syringe filter directly into 2 mL amber vials and cap each vial.
6. The extract is ready for injection into the LC-MS/MS system

Analytical Procedure - Liquid Chromatography / Mass Spectrometer Parameters

All participating laboratories have experience of analyzing TSNAs in smokeless products with LC-MS/MS and will use the mass spectrometer and parameters they generally use but with the UPLC or HPLC columns specified above in the Equipment part and the parameters and gradient specified below:

UPLC parameters

Column Temperature: 60.0 °C
Target Sample Temperature: 20.0 °C
Injection Volume: 10 µL
Flow rate: 0.45 mL/min
Mobile phase A: 10 mM ammonium acetate buffer, pH 4.7 (± 0.05)
Mobile phase B: 0.1 % (v/v) formic acid in acetonitrile
Gradient: Program the UPLC system according to table 3a

Table 3a. UPLC gradient

Time (min)	Flow rate (mL/min)	Mob. Ph. A (%)	Mob. Ph. B (%)	Gradient type
Initial	0.45	70	30	Initial
2.0	0.45	10	90	Linear
2.1	0.45	10	90	Linear
2.5	0.45	70	30	Linear
4.5	0.45	70	30	Linear

To achieve accurate quantification of the narrow UPLC peak widths, the number of data points collected for each peak should be 15 to 20.

HPLC parameters

Column Temperature: 60.0 ^{o}C
Target Sample Temperature: 20.0 ^{o}C
Injection Volume: 10 µL
Flow rate: 0.22 mL/min
Mobile phase A: Mill-Q water
Mobile phase B: 0.1 % (v/v) acetic acid in methanol
Gradient: Program the HPLC system according to table 3b

Table 3b. HPLC gradient

Time (min)	Flow (mL/min)	Mob. Ph. A (%)	Mob. Ph. B (%)	Gradient type
0	0.22	100	0	Initial
3.0	0.22	10	90	Linear
4.0	0.22	10	90	Linear
5.0	0.22	0	100	Linear
6.0	0.22	100	0	Linear
10.0	0.22	100	0	Linear

To achieve accurate quantification of the HPLC peak widths, the number of data points collected for each peak should be 15 to 20.

Mass Spectrometer Parameters

It is necessary that the Triple Quadrupole Mass Spectrometer has been carefully optimized for sensitivity of each analyte since some of the smokeless tobacco products contain low levels of TSNA.

Multiple Reaction Monitoring (MRM)

The quantification is done by using MRM-data of the transition of the precursor ion and the most abundant product ion. The quantification traces below (table 4) are recommended, but could be different for different systems. The dwell times need to have been optimized to achieve accurate quantification, the number of data points across each peak should be 15 to 20.

Table 4. Precursor and Product ions for MRM quantification of TSNAs

Name	Quantitation Trace (m/z)	Internal Standard Reference
NNK	208.2 > 121.9	NNK-d4
NNK-d4	212.2 > 126.1	N/A
NNN	178.1 > 148.0	NNN-d4
NNN-d4	182.1 > 152.1	N/A
NAT	190.1 > 160.0	NAT-d4

NAT-d4	194.1 > 164.0	N/A
NAB	192.1 > 162.0	NAB-d4
NAB-d4	196.1 > 166.1	N/A

System Suitability Testing

The system suitability must be performed for every sequence, both in the beginning and at the end of the sequence.

Sensitivity

First inject 2 prime samples and calibration standard nr. 1. Calculate the sensitivity of the system by calculating the signal to noise (S/N) for each analyte for calibration standard nr. 1. If S/N fall below 20 for any of the analytes, corrective actions must be taken before analyzing samples. The most common action to improve the S/N ration is to clean the source. Each participating laboratories should follow their in-house cleaning schemes. If the sensitivity of some laboratories instrument isn´t sensitive enough even if the instrument is newly cleaned and optimized, use the next level of the calibration standard and add a comment about that in the report spreadsheet.

Chromatographic performance

The performance of the chromatographic system is evaluated by inspecting the chromatogram of calibration standard nr. 1.

For the UPLC system

Visually inspect the peak shapes, if any of them seems to be tailing or fronting or if the peak width at 50 % peak height of any of the peaks are greater than 0.05 min, corrective action must be done before the sample could be analyzed. The criteria can probably be fixed by checking for dead volumes in the UPLC connections or replacing the UPLC column or the frit.

For the HPLC system

Visually inspect the peak shapes, if any of them seems to be tailing or fronting or if the peak width at 50 % peak height of any of the peaks are greater than 0.25 min, corrective action must be done before sample could be analyzed. The criteria can probably be fixed by checking for dead volumes in the HPLC connections or replacing the HPLC column or the guard column.

Sample Analysis

The priming sample is initially run (at least) two times followed by the 8 calibration standards and the QC 1 and QC 2. After every 10 sample replicates the QC standards are injected to enable the system suitability to be monitored. At the end of each sequence, the 8 calibration standards and the two QC standards should be analyzed again. An example of the run order is described below if all samples are prepared, extracted and analyzed the same day.

Quality control

The accuracy for each calibration point in the calibration curve and the QC standard should be between 85 – 115 %, except for the lowest calibration standard for which 80 – 120 % is acceptable.

QC limits are set at ± 15 % of the theoretical concentration. If any of the calibration- or QC standards fail, corrective action must be taken before reanalyzing the samples that were analyzed after the previously passed QC standard.

Calibration standard nr.1 is used both at the beginning and at the end of the sequence to check the sensitivity and chromatographic criteria specified above (System Suitability Testing).

Check that the blank (100 mM ammonium acetate) doesn't have any carry over after the strongest standard. If there is any carry over the injector may need a better washing step.

Reagent blanks should be included. They should be extracted and analysed as the samples in each analysis batch to secure that no contamination of TSNA occur from glassware, chemical reagents or instrumentation. The only exception for the reagent blank compared to the "real" samples are that the reagent blank sample extraction conical flask is empty until the internal standard and the extraction solution is added before extraction. If the reagent blank contains any TSNA, the contamination source has to be investigated and corrective action has to be taken. If the contamination has occurred from chemical reagents (e.g. the ammonium acetate buffer or the internal standard) all the samples in that batch have to be re-extracted and re-analyzed again using new chemical reagents without any contamination.

Extraction recoveries should be investigated in each analysis batch by preparing a spiked reagent blank. The spiked reagent blank is prepared by spiking an empty extraction conical flask with 20 µL of Intermediate Standard Solution nr 1. The spiked reagent blank will contain 800 ng NNN, NAT and NNK and 200 ng of NAB in the extraction conical flask. The spiked reagent blank is then treated and analyzed as the "real" samples (i.e. internal standard and extraction solution are added and the spiked reagent is extracted). The calculated concentration in the spiked reagent blank after analysis has to be compared to the theoretical concentration to determine the extraction recoveries. The extraction recovery should be in 85 – 115 % in each analysis batch. If a recovery sample fails, corrective action must be done before re-analyzing the samples.

The sample run order is as follows:

If all the samples could be prepared and analyzed during the same day

Blank (100 mM ammonium acetate).

Blank (100 mM ammonium acetate).

Calibration Standard 1

Calibration Standard 2

Calibration Standard 3

Calibration Standard 4

Calibration Standard 5

Calibration Standard 6

Calibration Standard 7

Calibration Standard 8

Blank (100 mM ammonium acetate).

QC standard 1

QC standard 2

Blank (100 mM ammonium acetate).

TSNA Standard Control (TSC)

TSNA Standard Control (TSC)

Blank (100 mM ammonium acetate).

Sample 1 (replicate 1 – 3)

Sample 2 (replicate 1 – 3)

Blank (100 mM ammonium acetate).

QC standard 1

QC standard 2

Sample 3 (replicate 1 – 3)

Sample 4 (replicate 1 – 3)

Blank (100 mM ammonium acetate).

QC standard 1

QC standard 2

Blank (100 mM ammonium acetate).

Sample 5 (replicate 1 – 3)

Sample 6 (replicate 1 – 3)

Blank (100 mM ammonium acetate).

QC standard 1

QC standard 2

Sample 7 (replicate 1 – 3)

Sample 8 (replicate 1 – 3)

Blank (100 mM ammonium acetate).

QC standard 1

QC standard 2

Sample 9 (replicate 1 – 3)

Blank (100 mM ammonium acetate).

Calibration Standard 1

Calibration Standard 2

Calibration Standard 3

Calibration Standard 4

Calibration Standard 5

Calibration Standard 6

Calibration Standard 7

Calibration Standard 8

Blank (100 mM ammonium acetate).

QC standard 1

QC standard 2

If the individual concentration of any TSNA analyte is higher than that of the highest calibration standard, the extracted sample should be diluted appropriately and reanalyzed to ensure that the TSNA concentration of the samples is within the calibration range.

Calibration

Set the quantitation method to perform a linear calibration (Y = kx + m) with the origin excluded and a weighting factor of $1/x^2$. Y is the response factor relative to the internal standard and x is the concentration (ng/mL) of a standard. All eight calibration standards, both at the beginning and at the end of the sequence, should be included in the calibration curve. A new calibration curve must be acquired daily. Fresh aliquots of TSNA calibrating standards, Cal 1 through Cal 8, must be used.

Calculations

A calibration graph of response ratio of each TSNA / labelled TSNA (e.g. NNN /NNN-d4) versus concentration is plotted. Acceptable R^2 –values from the calibration graph must be ≥ 0.995. The accuracy for each calibration point in the calibration curve and the QC standard should be between 85 -115 % except for the lowest calibration standard where 80 – 120 % is acceptable. All calibration and sample analysis calculations utilize Relative Response Factors:

$$RRF = \frac{A_a}{A_{IS}} * C_{IS}$$

Where:
RRF = Relative response factor
A_a = Area of the target analyte
A_{IS} = Area of the corresponding internal standard
C_{IS} = Concentration of the corresponding internal standard

The concentration of the target analytes in a sample (ng/g), is determined using the calculated RRF for the sample, the slope and intercept obtained from the appropriate calibration curve, and the equation:

$$Conc(wet) = \frac{RRF - Int}{slope} * \frac{V}{M}$$

Where:
Conc (wet) = Calculated concentration (ng/g)
Int = y-intercept from the calibration curve
Slope = Slope from the calibration curve
V= Final volume of the extraction solution (mL).
M= Weight of the smokeless tobacco product sample (g).

Results report

In each laboratory the measurements shall be carried out by one operator selected as being representative of those likely to perform the measurements in normal operations. Comments and *deviation from the documented analytical procedure*, or other relevant observations shall be reported in the spreadsheet.

The individual test results are entered in the provided spreadsheet. The test results shall be reported with at least three decimals. The equipment used should be noted in the result report.

The completed spreadsheet should be returned to Linda Drake, BAT GR&D
Linda_Drake@bat.com

Any questions or concerns regarding this TSNA method should be addressed to Johan Lindholm, Swedish Match North Europe
johan.lindholm@swedishmatch.se

CSTS Collaborative Study 2009
Technical Report

Appendix 4

CORESTA Smokeless Tobacco Study (CSTS) 2009

Hauleithner Alexander, JTI R&D Oekolab

Data analysis of the CORESTA Smokeless Tobacco 2009 Collaborative study was performed following the statistical model provided by ISO 5725-2 (1994) "*basic method for the determination of repeatability and reproducibility of a standard measurement method*". Additionally, z-scores were derived to check LAB performances.

9 smokeless tobacco products were assessed for their content in water (before and after analysis), moisture, pH, nicotine and (sum of) TSNAs. 23 laboratories participated in the study.

The following tables list the participating laboratories and the samples analyzed in the current collaborative study. Please note that the laboratories are listed in alphabetical order and that this does not correspond to the LAB Codes used in this report (LAB Codes 1-23).

Table 1: Laboratories participating.

List of Participating Labs	
A ANDRE	KT&G
ARISTA	LABSTAT
BAT HOP	PMI
BAT GR&D	POESCHL
BORGWALDT	RJRT
CNTC	SEITA IMPERIAL
CONWOOD	SMNA
EUROFINS	SMNE
GLOBAL LABS	SWISHER
ITC	UNIV KENTKY
ITG REEMTSMA	USSTMC
JTI AUSTRIA	

Table 2: Sample types and sample codes.

Sample Type	Sample Code
Nasal Snuff	1
Loose Snus	2
Chewing Tobacco - Twist	3
Chewing Tobacco - Flake	4
Pellet	5
Chewing Tobacco - Loose Leaf	6
Moist Snuff	7
Moist Snuff	8
Pouched Snus	9

Table 3 summarizes analytes, some methods and laboratories participating at the CORESTA Smokeless Tobacco 2009 Collaborative study.

Table 3: Analytes, methods and laboratories participating at the CORESTA ST 2009 Collaborative Study.

		Laboratory																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Water before (%)	GC	X		X				X	X	X			X											
	KF		X		X						X	X		X	X		X	X	X	X	X		X	
	NIR																							X
Water after (%)	GC	X		X				X	X	X			X			X								
	KF		X				X				X	X		X	X		X	X	X	X	X		X	
	NIR																							X
Moisture		X	X	X	X	X	X	X	X	X	X		X	X	X	X		X		X	X	X	X	X
pH		X	X	X	X	X	X	X	X	X	X		X	X	X	X	X		X	X	X	X	X	X
Nicotine		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
TSNA	sum	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	NNN	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	NAT	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	NAB	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	NNK	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Standard	X	X	X	X		X	X	X	X	X		X	X	X			X				X		X

For the determination of water content (% before and after analysis) two methods were recommended: “Determination of Water in Tobacco and Tobacco Products by Karl Fischer Method” (CORESTA Recommended Method N°56) and/or “Determination of Water in Tobacco and Tobacco Products by Gas Chromatographic Analysis” (CORESTA Recommended Method N°57).

LAB 23 used an alternative method for the determination of water content (NIR transmittance). An r&R analysis was performed with all laboratories and all three methods included. Additionally, for the two recommended methods two separate r&R analysis were performed.

The moisture of the nine samples was given in percent. LABs 11, 16 and 18 did not deliver data for moisture.

The pH was measured after 5, 15 and 30 minutes of extraction time. A possible change in pH depending on the extraction time was checked. Statistical analysis was performed for separate methods (i.e. CDC and CDC?). Laboratory 6 used another method and was excluded from analysis. LABs 11 and 17 did not deliver data.

To measure the content of nicotine laboratories used different types of methods. These were grouped into three groups: CRM62, CDC and OWN. Separate r&R figures were derived for each of the groups.
To measure the sum of TSNAs in the samples laboratories also used different methods. An overall r&R result was derived, and additionally separate r&R figures for the method groups: LCMSMS SMNE, LCMSMS and GC.

15 laboratories reported results for a TSNA standard that was sent out to the participating laboratories. The results were used to correct the reported TSNA results for the difference between the laboratories’ calibration standards. Therefore, for TSNAs two results were calculated, “*TSNAs as received*” and “*TSNAs corrected*”. The correction factors are listed later in this report.

The analysis comprises three steps:

Plots 1-6 show the raw data for the six analytes under consideration. The raw data was grouped by methods as appropriate. The line displays the overall mean of the

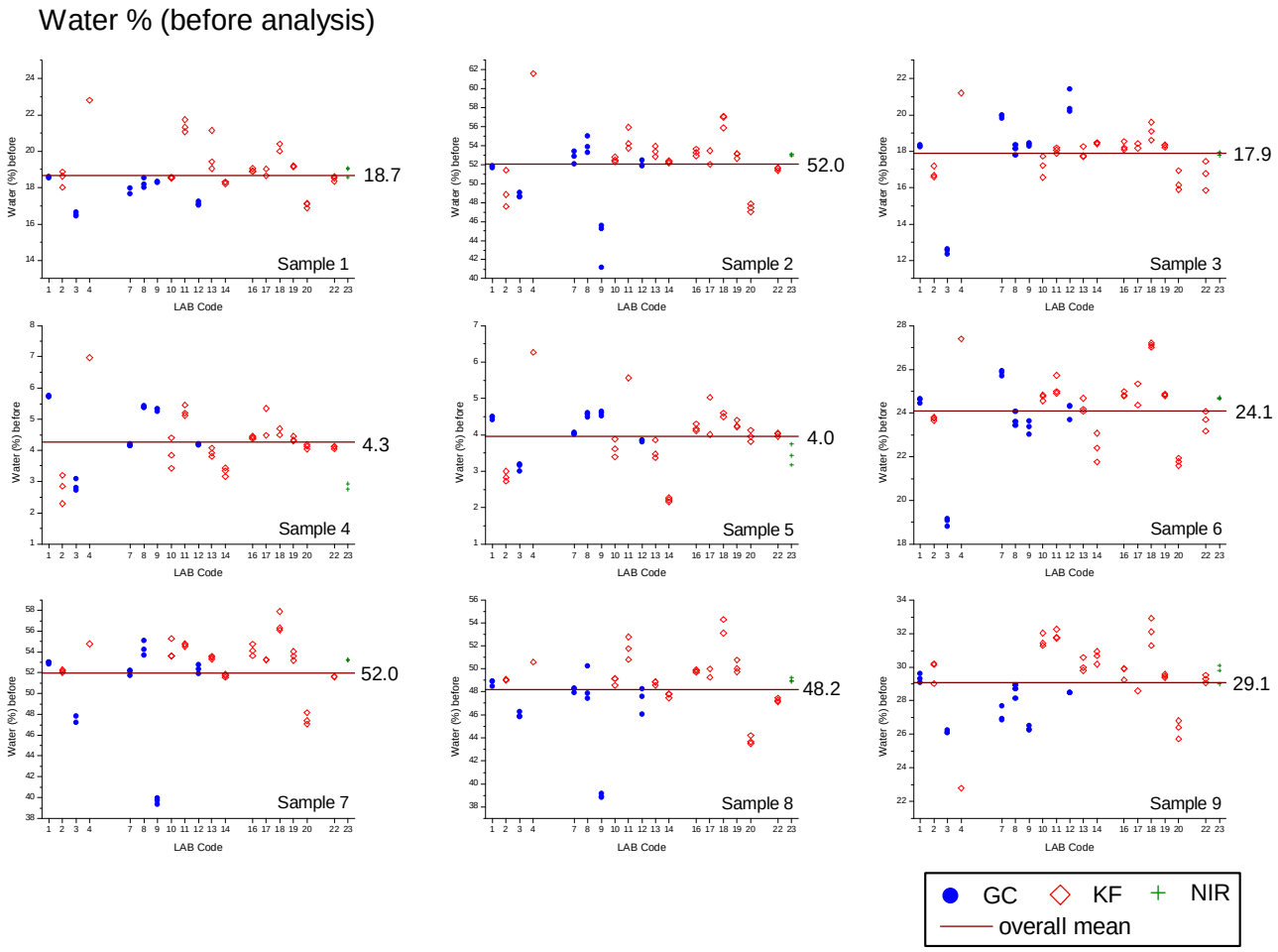
data reported. At this stage no outliers were excluded. The raw data plots start on page 4.

Tables of means and standard deviations for the analytes are listed in an appendix to this report.

As per ISO 5725-2, data consistency was checked using graphical and numerical outlier detection techniques. A short description of the procedures employed is given on page 10.

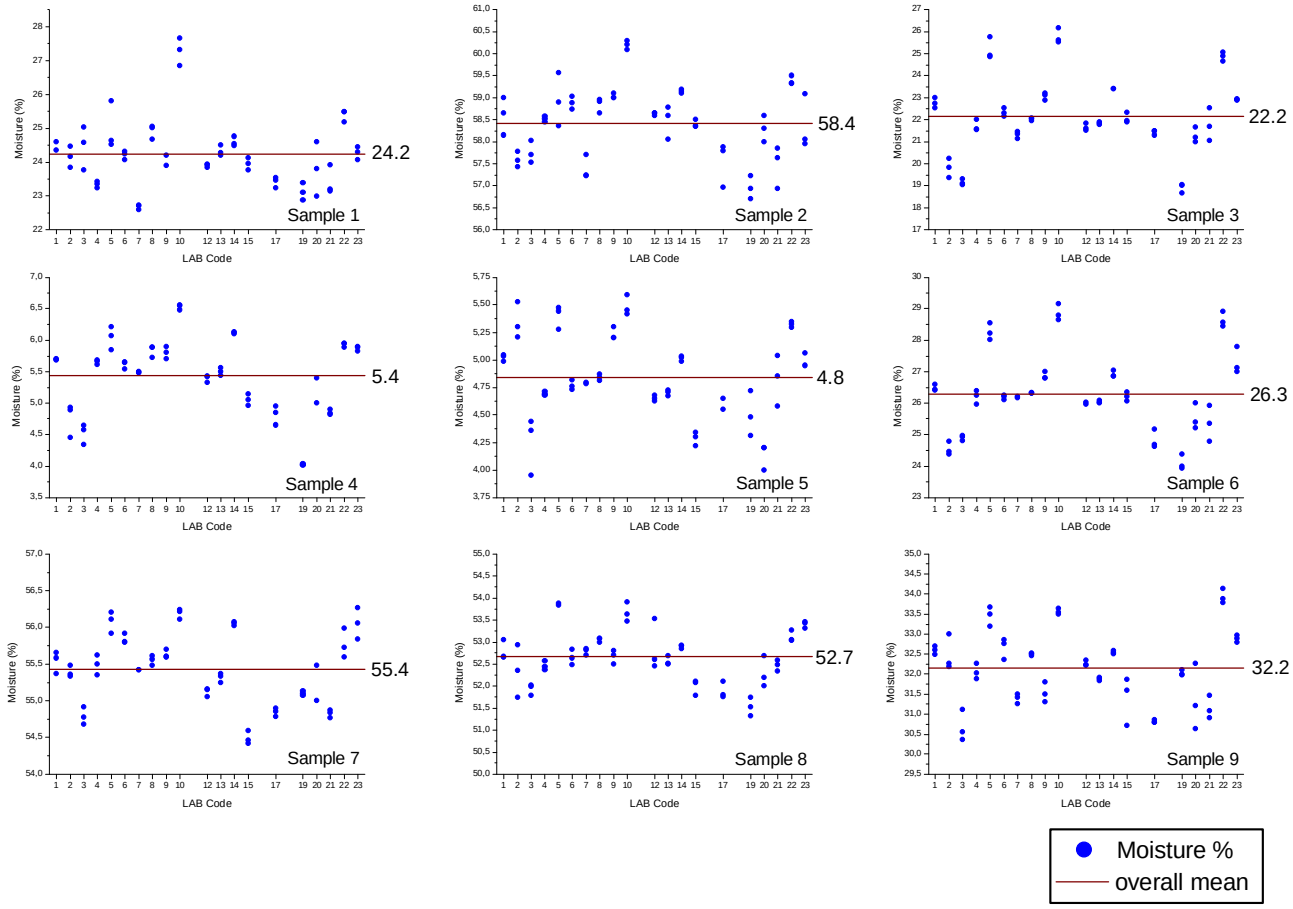
Finally and after removal of outlying data, the final mean, repeatability standard deviation (r SD), reproducibility standard deviation (R SD), repeatability (r) and reproducibility (R) for each analyte yield were calculated from the remaining data.

Additionally, *z*-scores were derived. A *z*-score indicates how many standard deviations an observation (a laboratories result) is above or below the mean. Outlying data was removed prior to the calculation of the *z*-scores.
The *z*-scores were used to derive an overall performance of a laboratory for a parameter (and method used). The z-score evaluation starts on page 56.

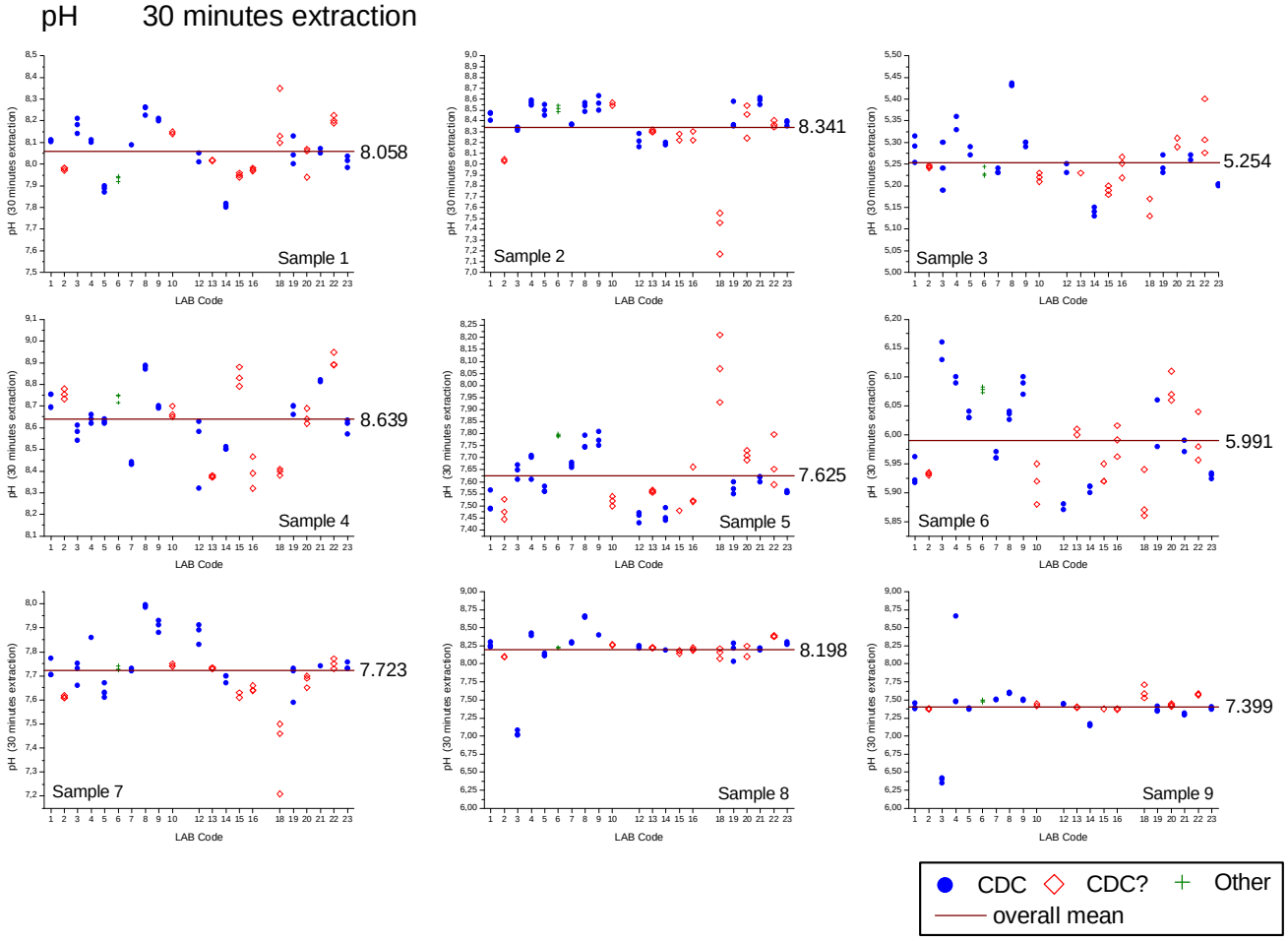


Plot 1: Raw data plots for Water % (before analysis). Three different method groups.

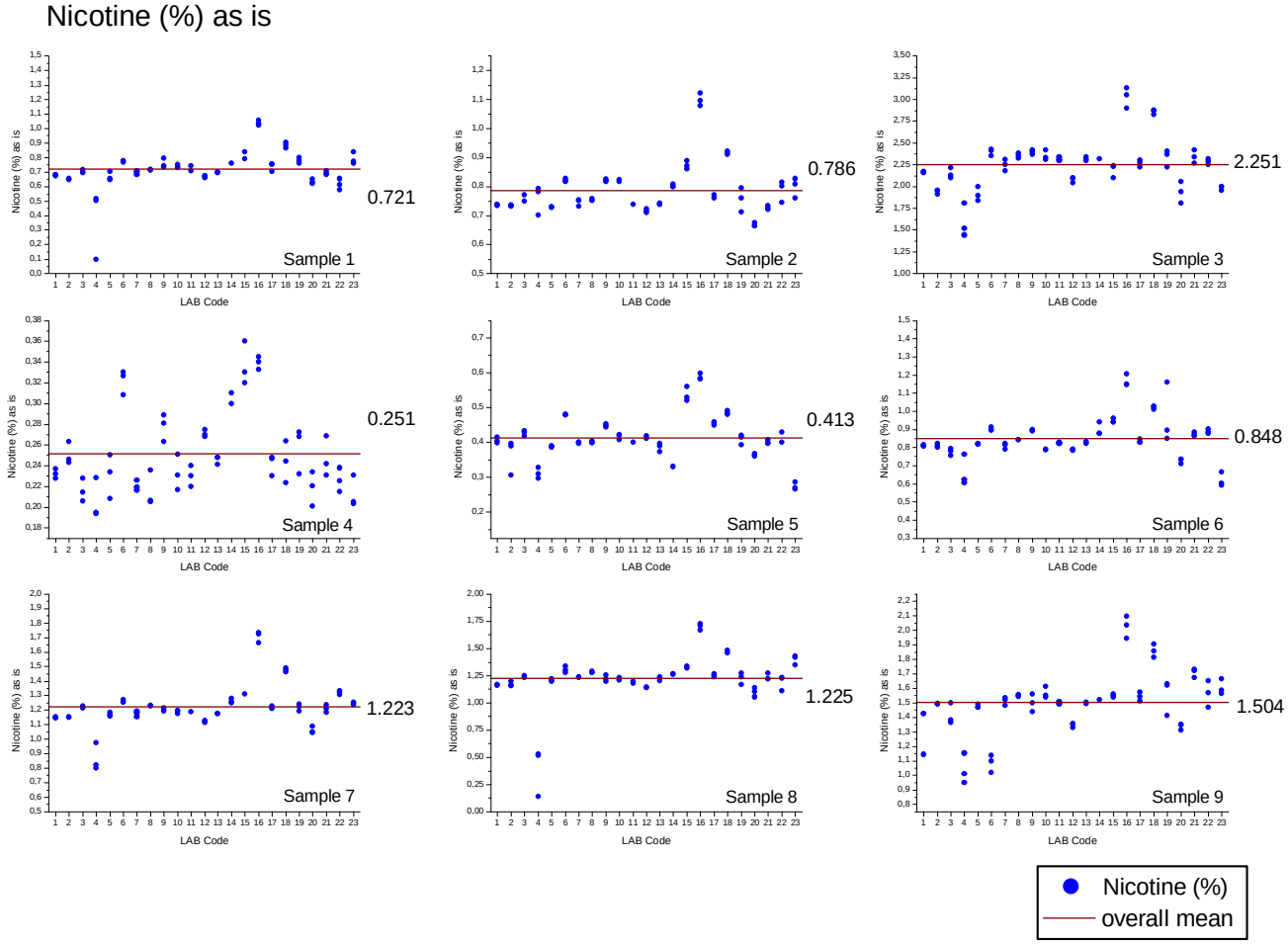
Moisture %



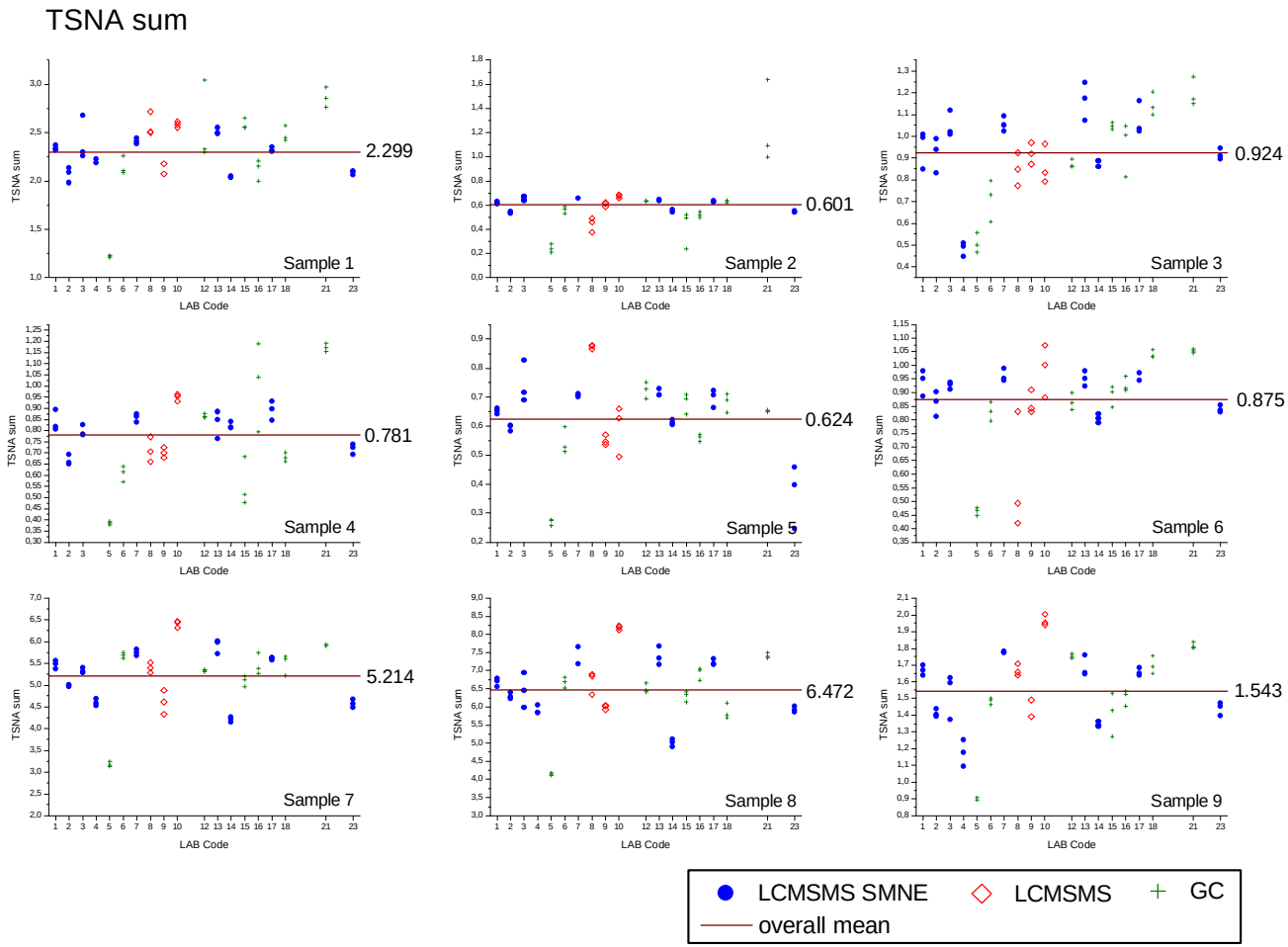
Plot 2: Raw data plots for Moisture %.



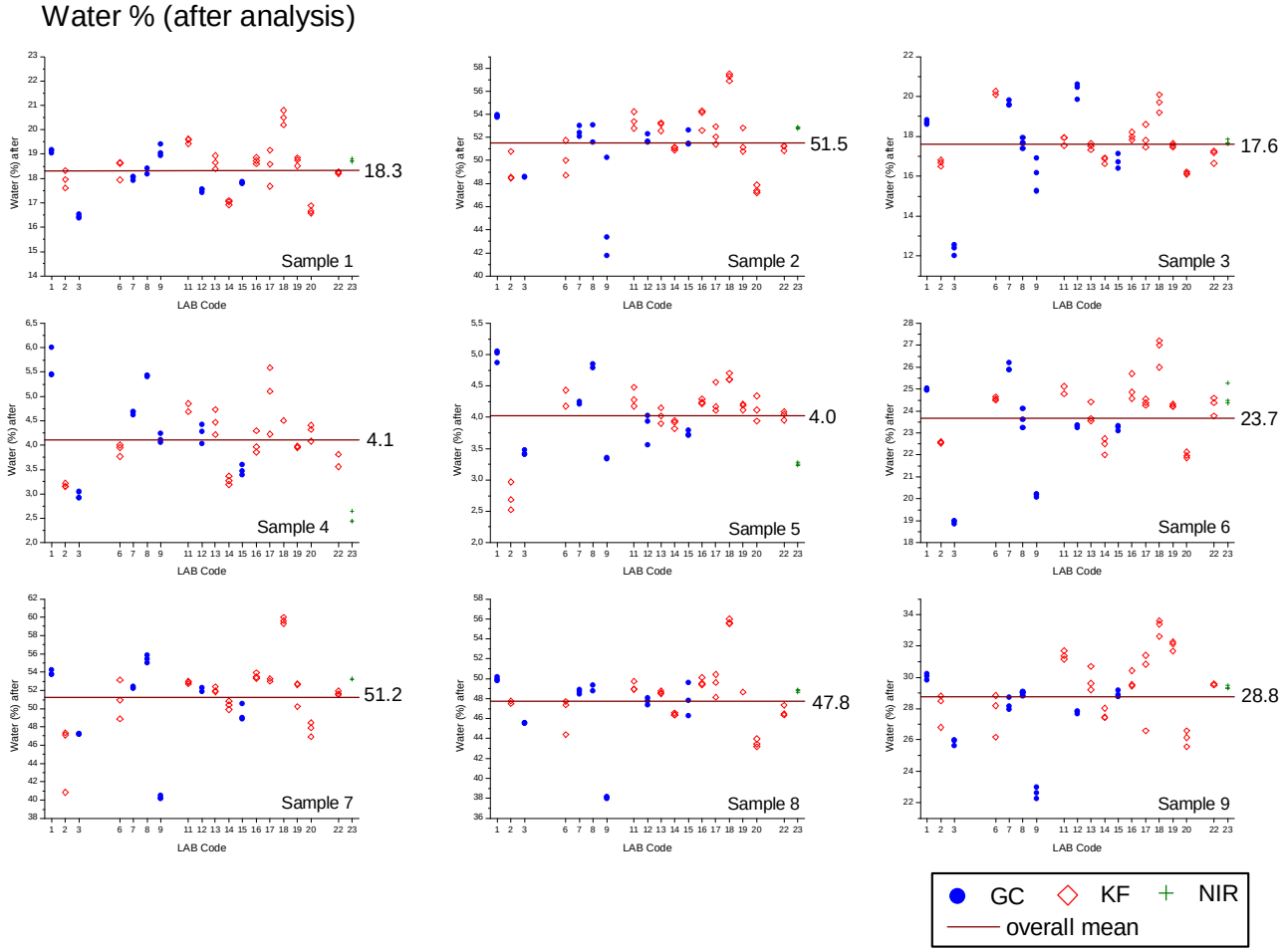
Plot 3: Raw data plots for pH after 30 minutes extraction time. Three different method groups.



Plot 4: Raw data plots for Nicotine (%) as is.



Plot 5: Raw data plots for TSNA sum. Three different method groups.



Plot 6: Raw data plots for Water % (after analysis). Three different method groups.

The raw data indicated that it was suitable for further investigation, with single laboratories' results lying further away from the overall mean than others or exhibiting a higher variation. Therefore, and as recommended in ISO 5725-2, data consistency techniques were employed on the raw data.

Table 4 lists the data consistency techniques proposed in ISO 5725-2 and applied subsequently on the raw data of the current proficiency test.

Table 4: Data consistency techniques proposed in ISO 5725-2.

data consistency	graphical	numerical
between laboratories	MANDEL's *h*	GRUBBS' single outlier
within an laboratory	MANDEL's *k*	COCHRAN's C test

In a first step raw data was examined using the two graphical outlier detection techniques. MANDEL's *h* plots check the between laboratory consistency by comparing the overall mean results. In the single graphs, lines were drawn indicating 0.95 straggling and 0.99 outlying limits. The *h*-plots start on page 11.

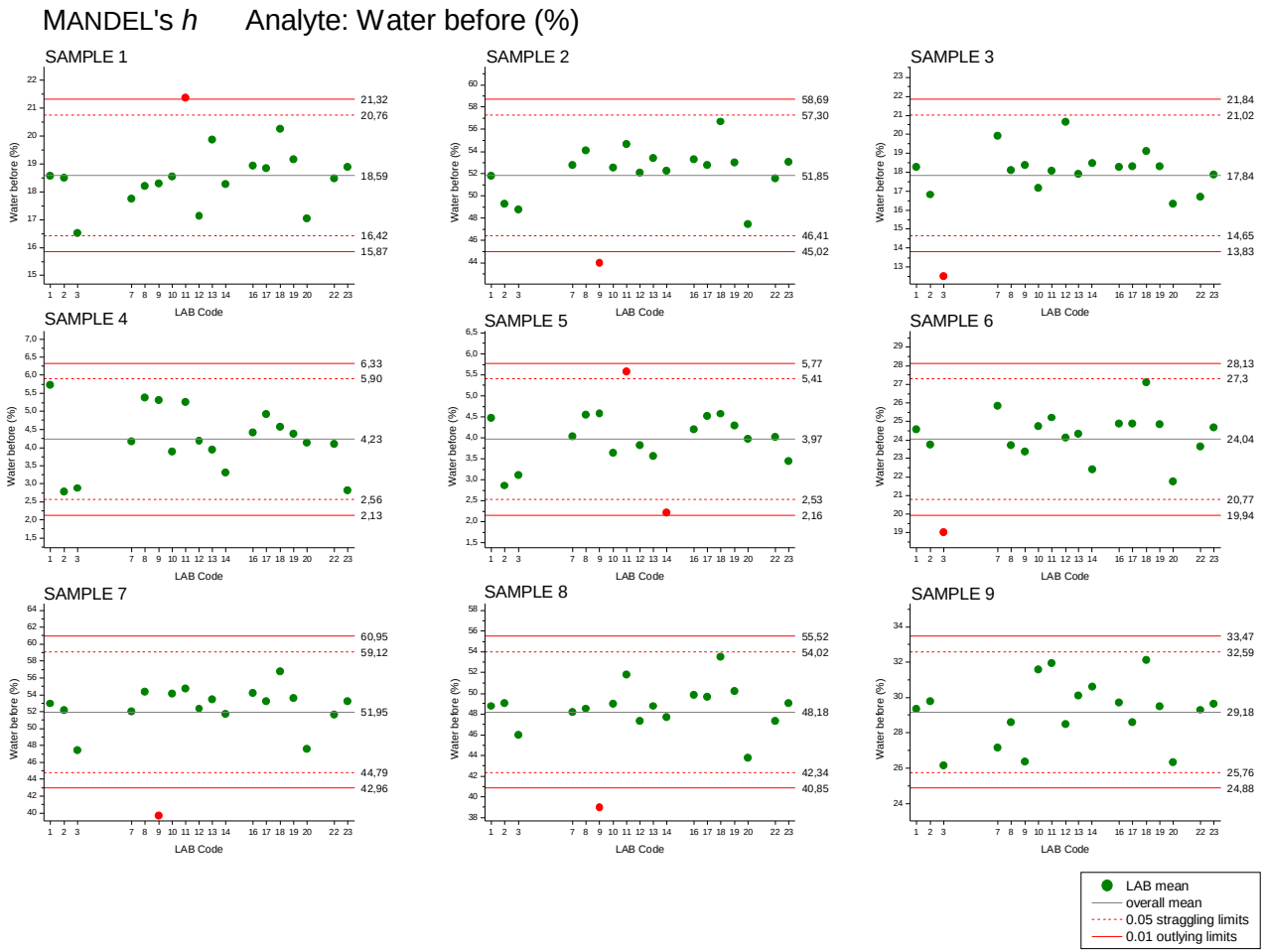
MANDEL's *k* plots check the consistency within laboratories. The laboratories' standard deviations for each level were compared against straggling and outlying limits. Large *k*-values indicate a poorer repeatability compared with other laboratories. The *k*-plots start on page 17.

Please note that in MANDEL's *h* and *k* plots not the actual h and k values were displayed but their corresponding values for the mean and standard deviation. This was done to facilitate data evaluation.

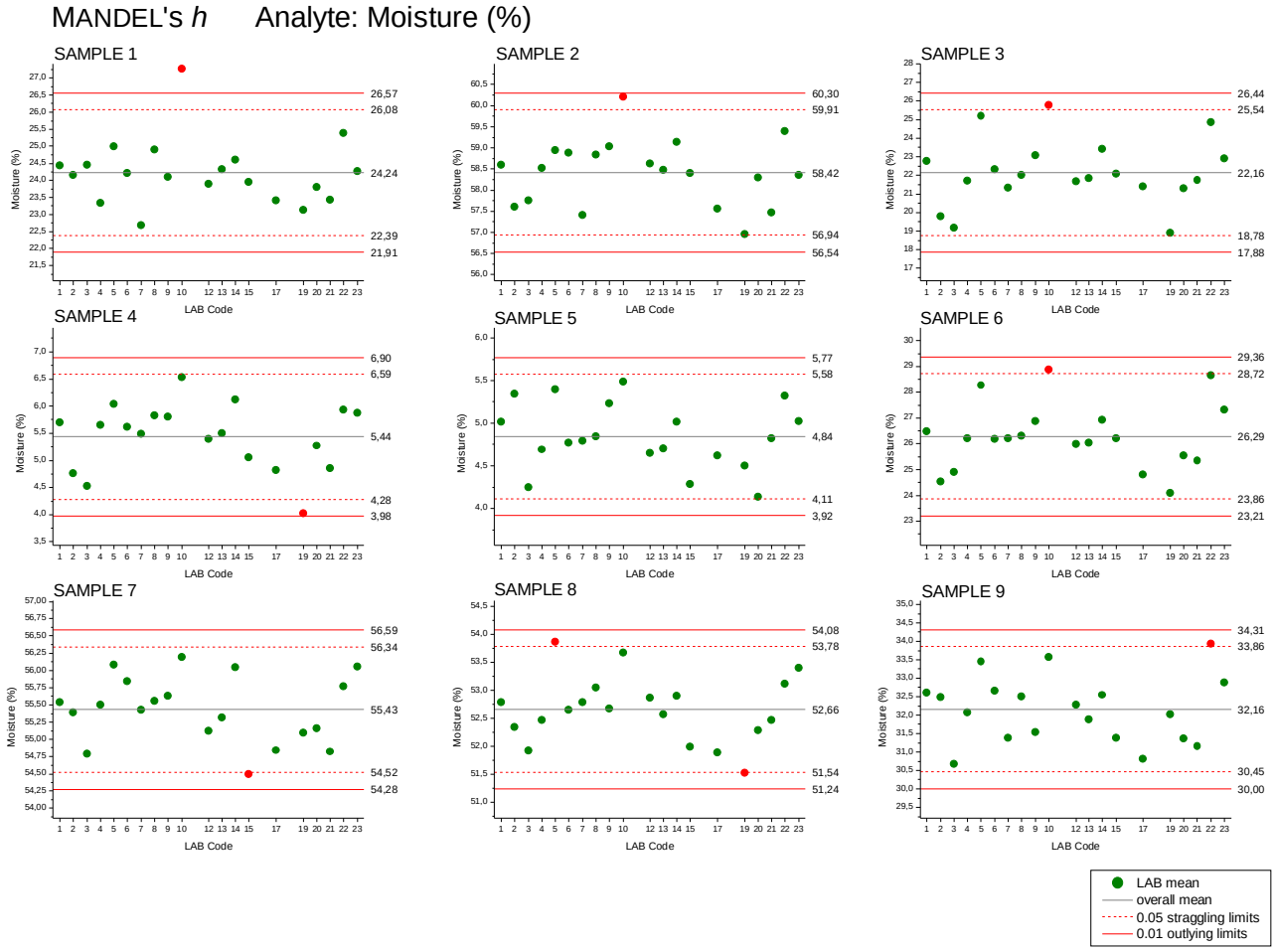
In both, MANDEL's *h* and *k* plots, observations beyond straggling and outlying limits were suspicious ones and marked in red colour to facilitate detection.

Both graphical outlier detection techniques are more likely than their corresponding numerical outlier detection techniques. To avoid excessive data exclusion, no final decisions were made at that stage. Nevertheless, the plots should serve in laboratory evaluation.

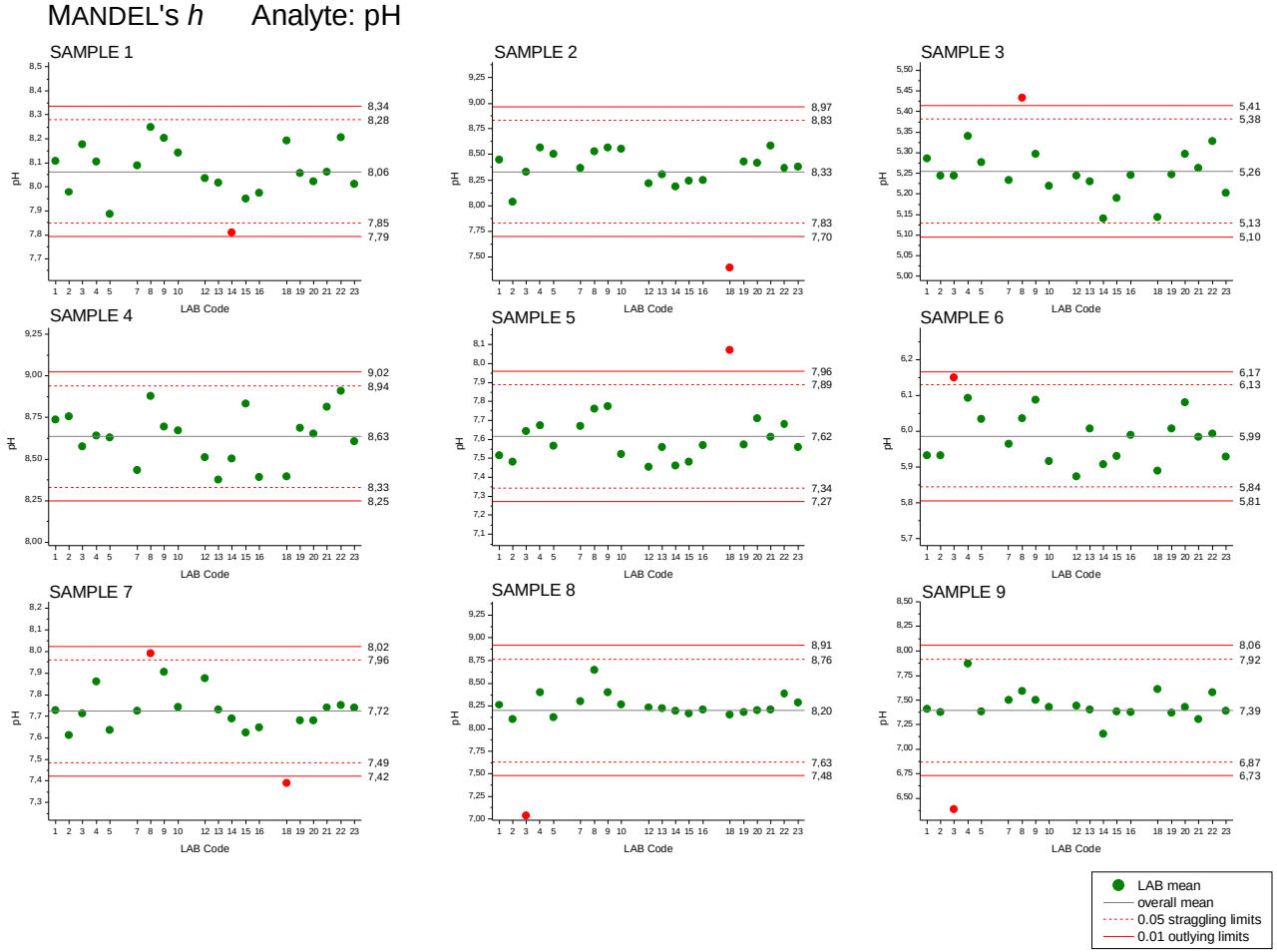
In the following plots dotted lines represent the 0.95 straggling limits and straight lines the 0.99 outlying limits.



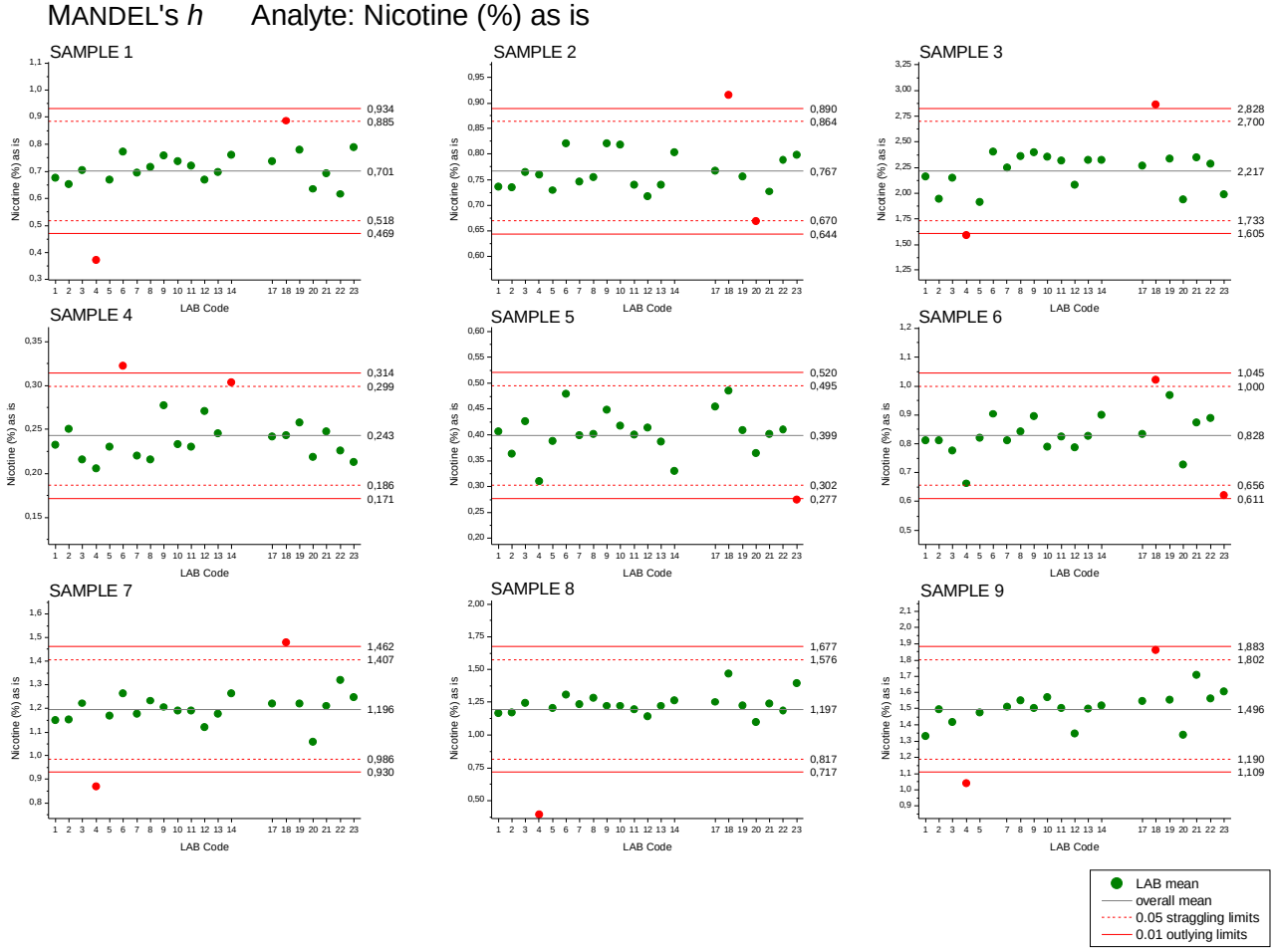
Plot 7: MANDEL's *h* plot for Water before (%). Samples 1-9.



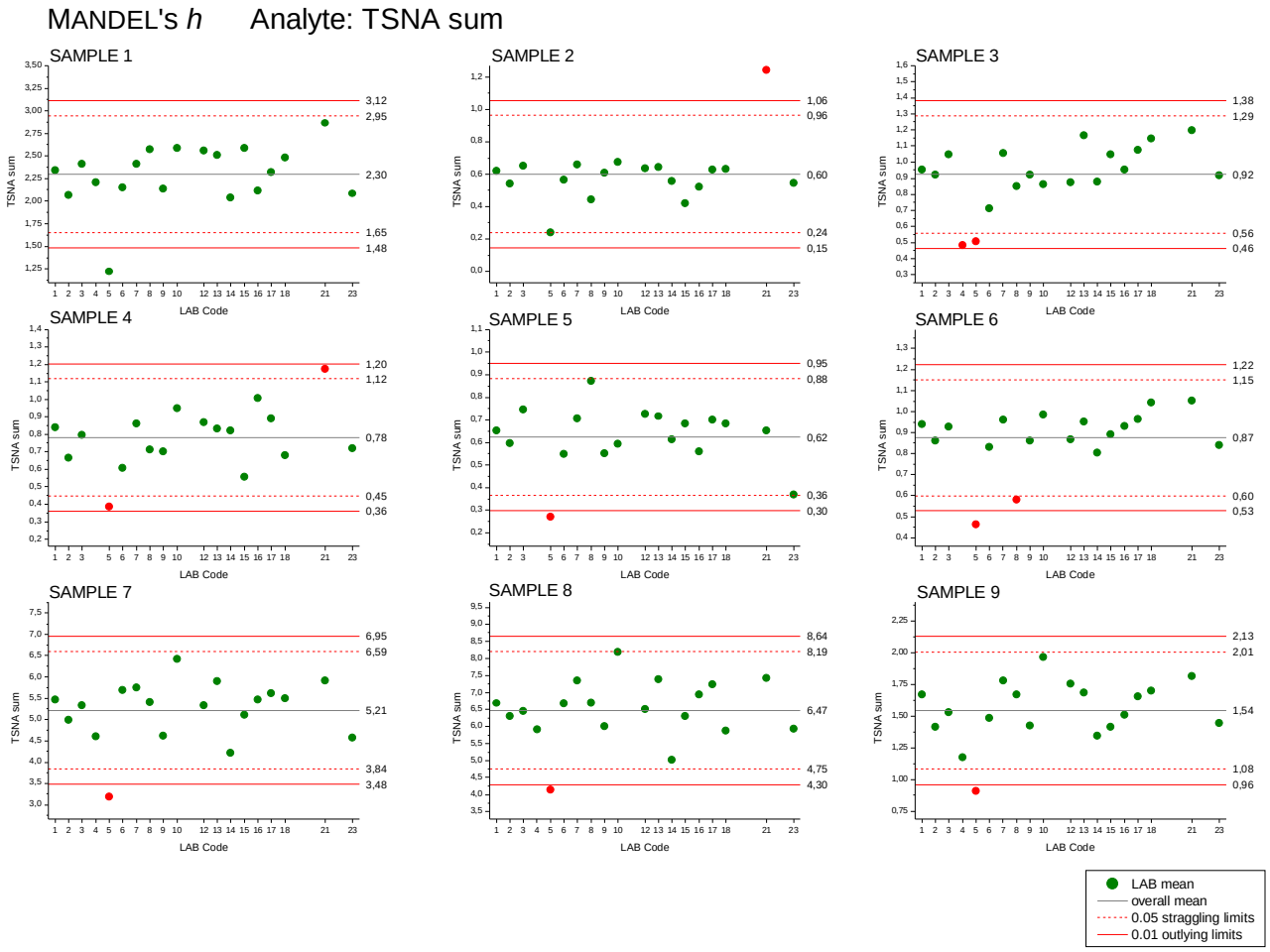
Plot 8: MANDEL's *h* plot for Moisture (%). Samples 1-9.



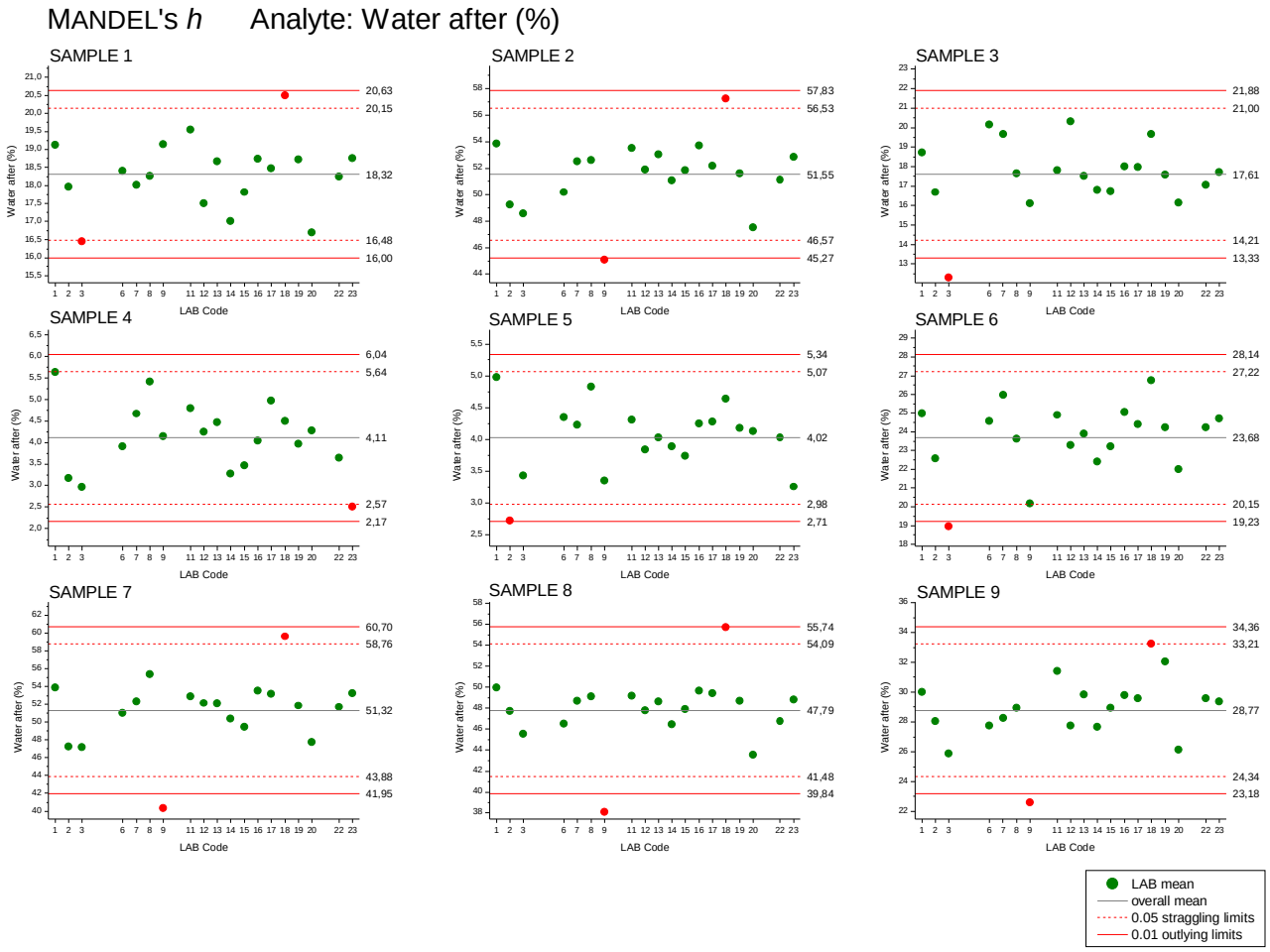
Plot 9: MANDEL's *h* plot for pH. Samples 1-9.



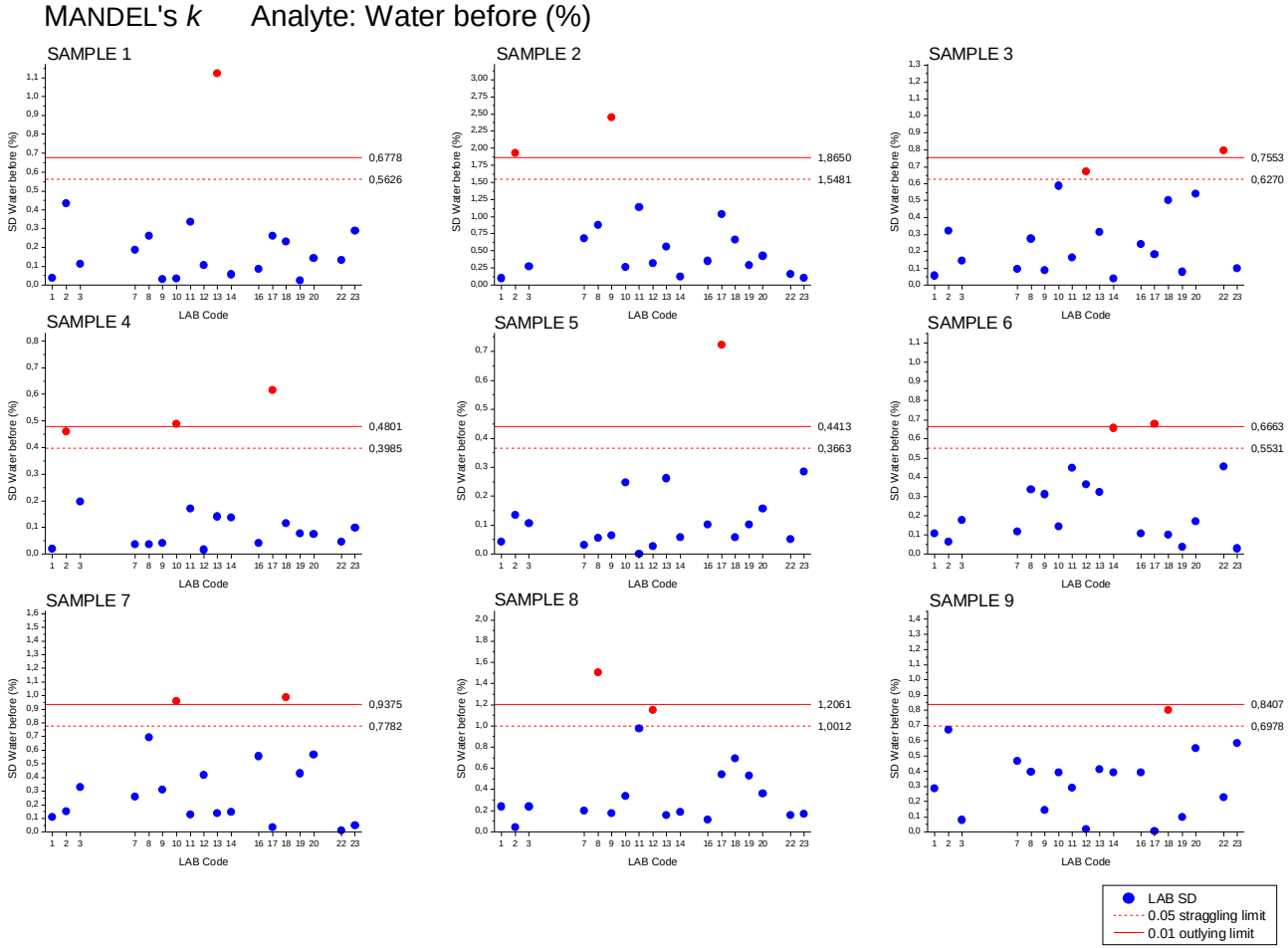
Plot 10: MANDEL's *h* plot for Nicotine (%) as is. Samples 1-9.



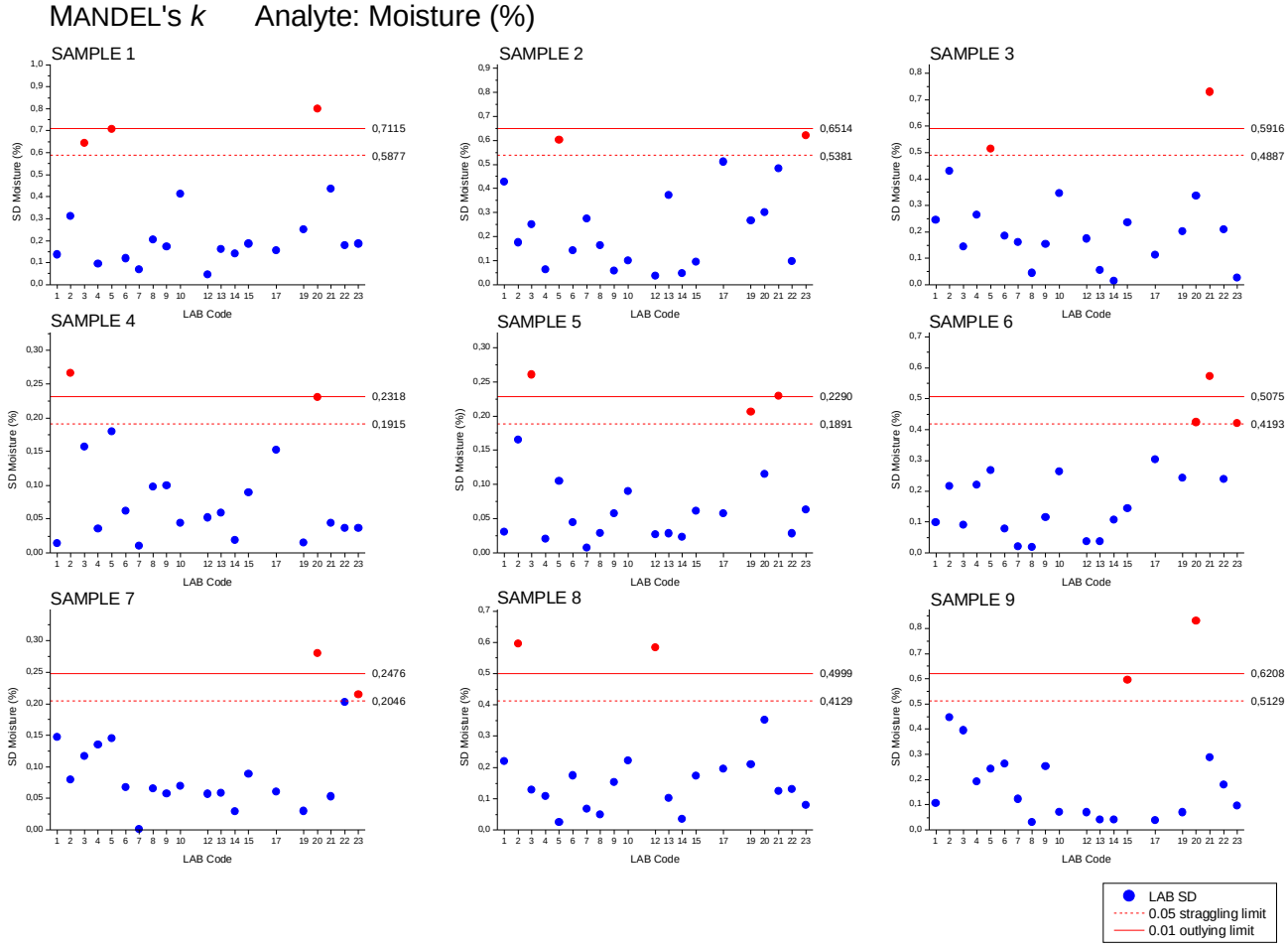
Plot 11: MANDEL's *h* plot for TSNA sum. Samples 1-9.



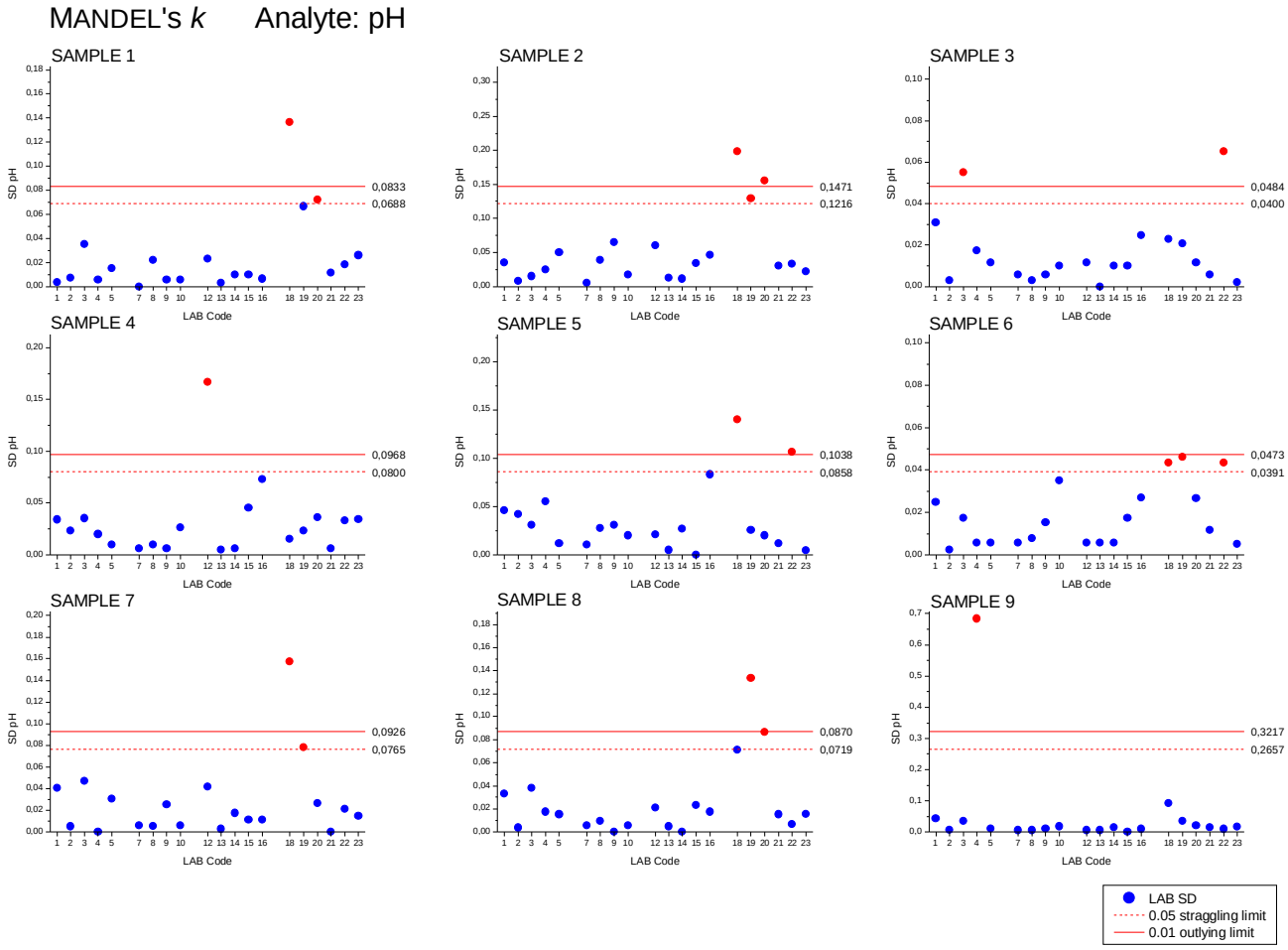
Plot 12: MANDEL's *h* plot for Water after (%). Samples 1-9.



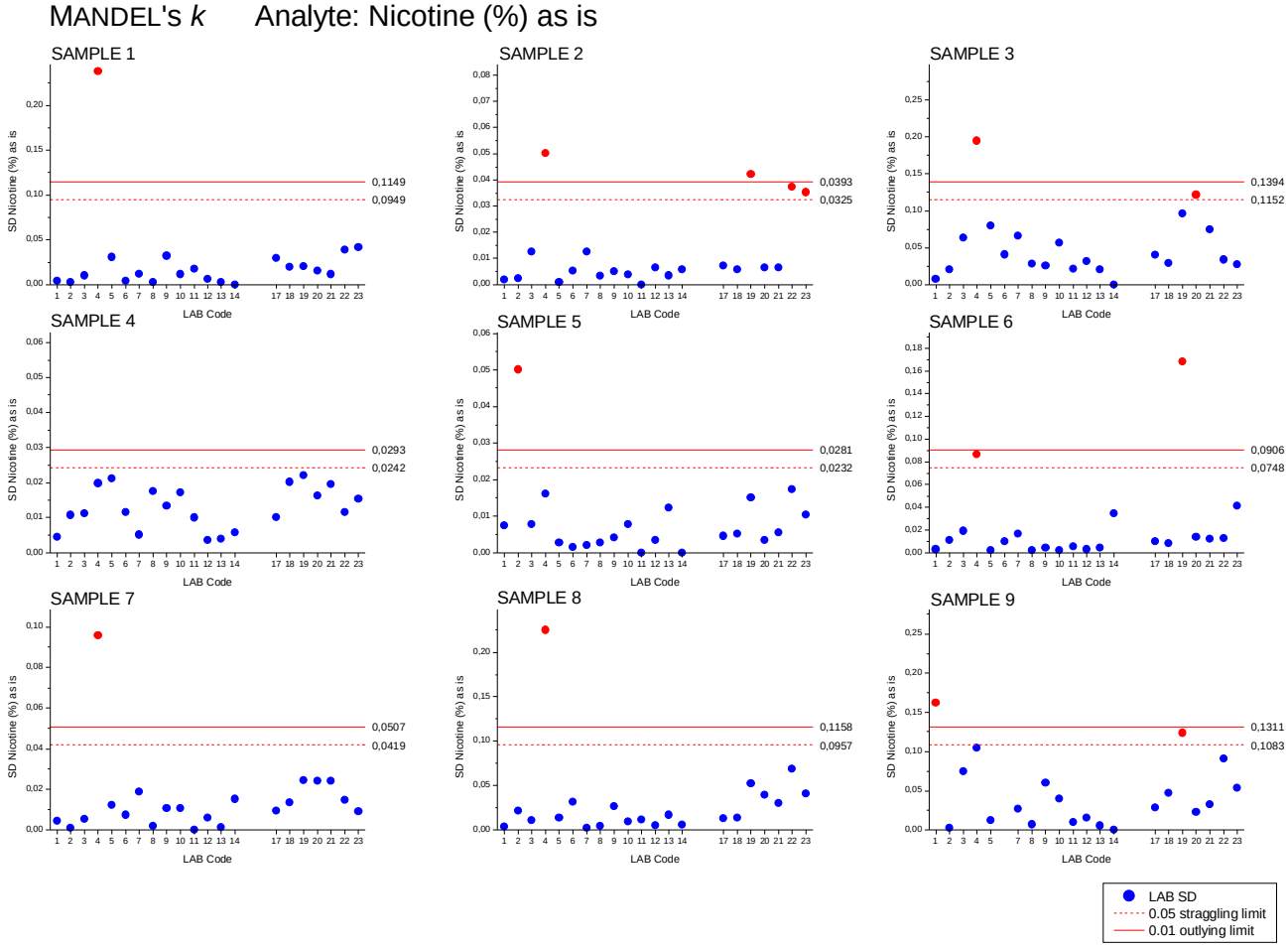
Plot 13: MANDEL's *k* plot for Water before (%). Samples 1-9.



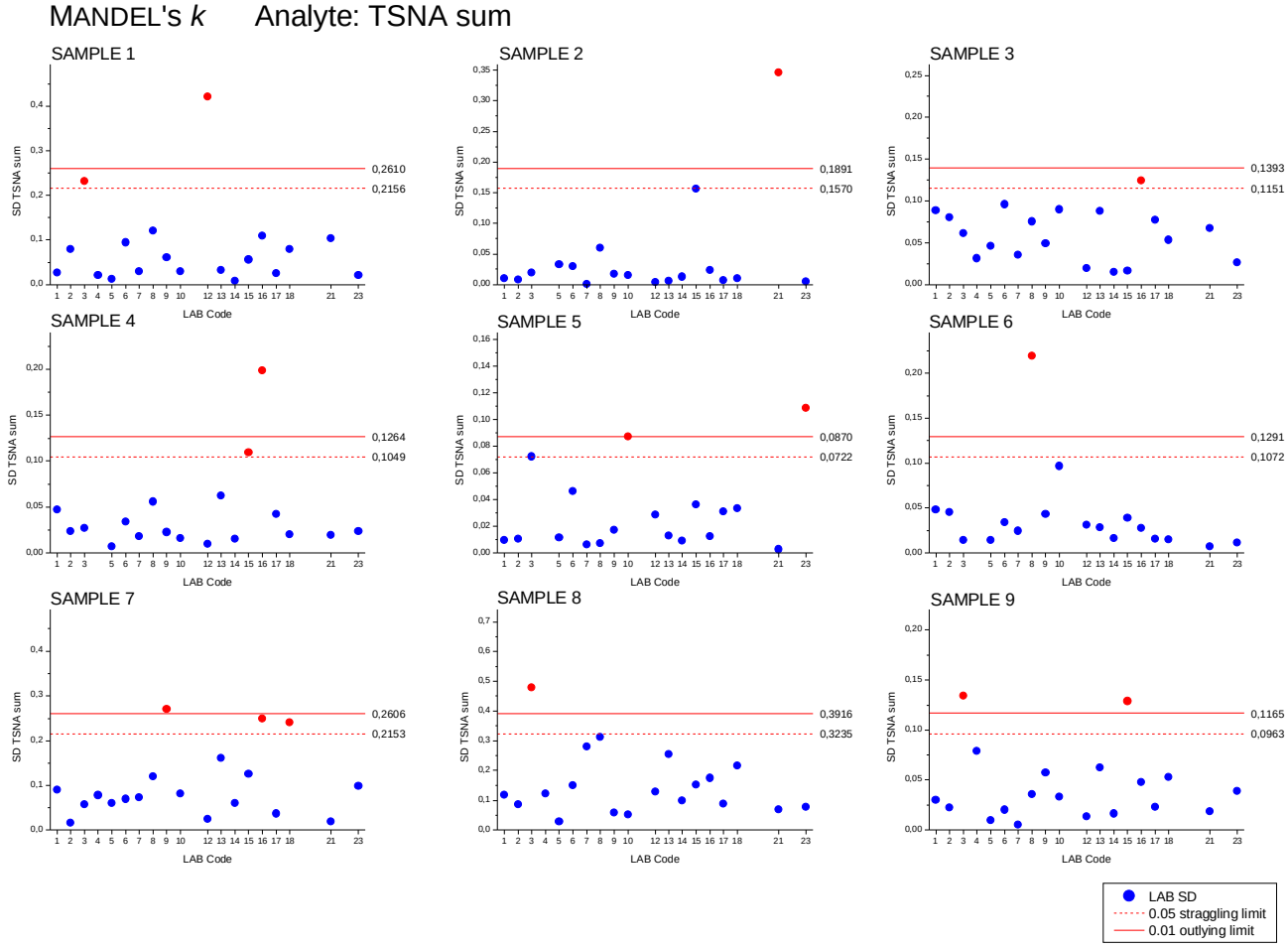
Plot 14: MANDEL's *k* plot for Moisture (%). Samples 1-9.



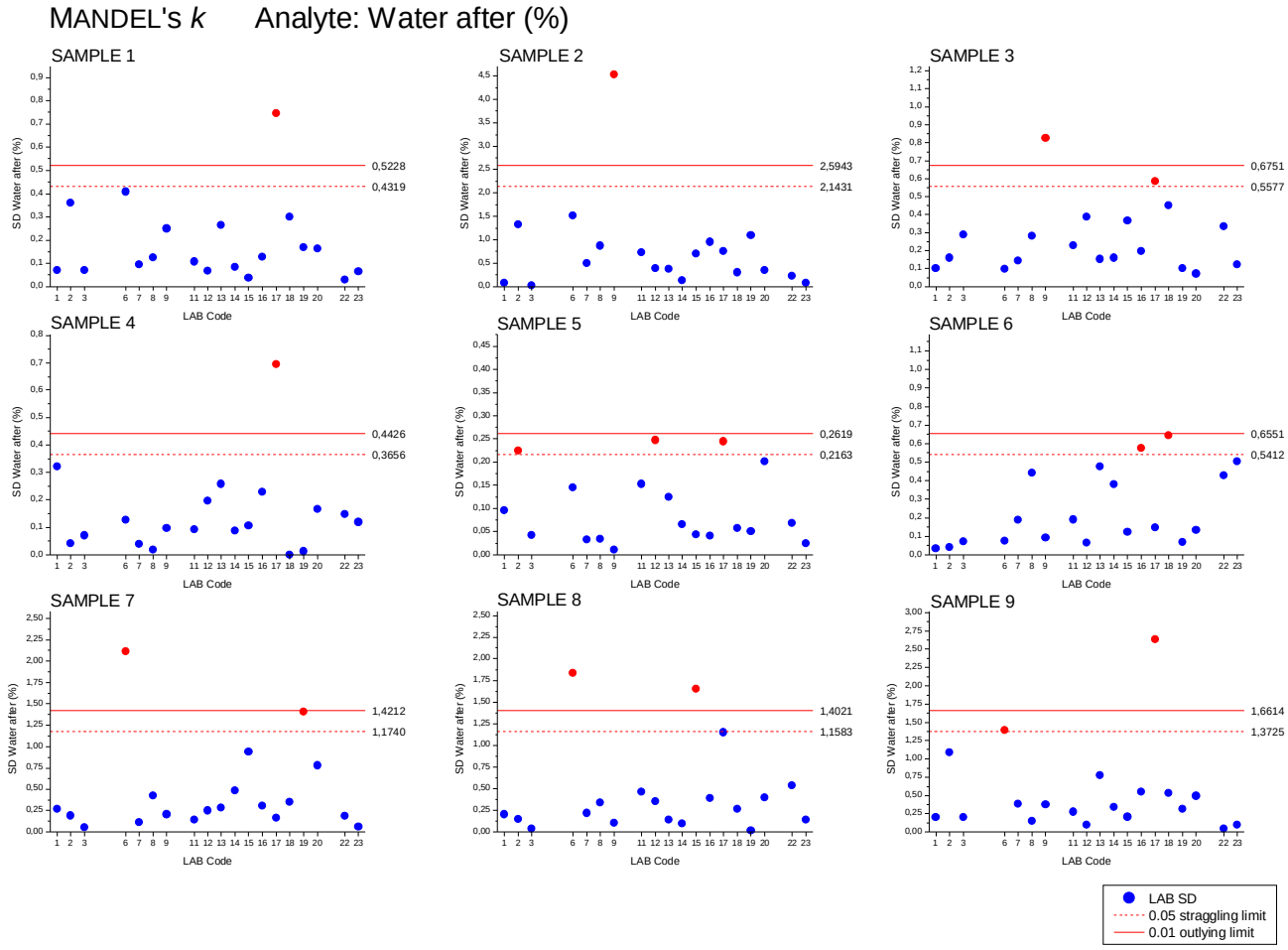
Plot 15: MANDEL's *k* plot for pH. Samples 1-9.



Plot 16: MANDEL's *k* plot for Nicotine (%) as is. Samples 1-9.



Plot 17: MANDEL's *k* plot for TSNA sum. Samples 1-9.



Plot 18: MANDEL's *k* plot for Water after (%). Samples 1-9.

The outlier detection continued with numerical methods.

COCHRAN's test is a test of the within laboratory consistency and was applied first. The test was applied in iterations and continued as long as no further outlying data could be detected.

Note that COCHRAN's criterion tests only the highest value in a set of variances and is therefore a one-sided outlier test. Heterogeneity of variances may also occur with standard deviations comparatively too small. However, it seems unreasonable to reject data from a laboratory because it has accomplished a higher precision than the other. Hence, COCHRAN's test is considered adequate.

Subsequently, GRUBBS' test was performed.

Outliers detected with numerical methods were excluded from analysis, all stragglers found were retained.

The following table summarise all outlying and straggling laboratories detected with graphical and numerical outlier detection methods.

Table 5: Summary of outliers and stragglers detected with graphical and numerical outlier detection methods.

		MANDEL's *h*		MANDEL's *k*		COCHRAN's		GRUBBS'	
		OUTLIER 0.01	STRAGGLER 0.05	OUTLIER 0.01	STRAGGLER 0.05	OUTLIER 0.01	STRAGGLER 0.05	OUTLIER 0.01	STRAGGLER 0.05
Sample 1	Water before	11		13		13			
	Moisture	10		20	3 5		20	10	
	pH		14	18	20	18 19 20			
	Nicotine	4	18	4		4		4	
	TSNA sum	5		12	3	3 12		5	
	Water after		3 18	17		17			
Sample 2	Water before	9		2 9		2 9			9
	Moisture		10		5 23				
	pH	18		18 20	19	18 19 20		18	
	Nicotine	18	20	4 19	22 23	4 19 23			18
	TSNA sum	21		21		8 15 21		21	
	Water after	9	18	9		9			
Sample 3	Water before	3		22	12			3	
	Moisture		10	21	5		21		
	pH	8		3 22		3 22			
	Nicotine	4 18		4	20	4			
	TSNA sum		4 5		16				
	Water after	3		9	17				3
Sample 4	Water before			10 17	2	2 10 17			
	Moisture		19	2	20		2		
	pH			12		12	16		
	Nicotine	6	14						
	TSNA sum		5 21	16	15	15 16			
	Water after		23	17		17			
Sample 5	Water before		11 14	17		17			
	Moisture			3 21	19		3		
	pH	18		18 22		16 18 22		18	
	Nicotine	23		2		2			
	TSNA sum	5		10 23		3 10 23			
	Water after		2		2 12 17				
Sample 6	Water before	3		17	14				3
	Moisture		10	21	20 23		21		
	pH		3		18 19 22				
	Nicotine	18 23		19	4	4 13 19 23			
	TSNA sum	5	8	8		8 10			5
	Water after	3			16 18				

Table 5 continued.

		MANDEL's *h*		MANDEL's *k*		COCHRAN's		GRUBBS'	
		OUTLIER 0.01	STRAGGLER 0.05	OUTLIER 0.01	STRAGGLER 0.05	OUTLIER 0.01	STRAGGLER 0.05	OUTLIER 0.01	STRAGGLER 0.05
Sample 7	Water before	9		10 18				9	
	Moisture		15	20	23		20		
	pH	18	8	18	19	18 19			
	Nicotine	4 18		4		4			4
	TSNA sum	5		9	16 18				5
	Water after	9	18	6	19	6 19	15		9
Sample 8	Water before	9		8	12	8	12	9	
	Moisture		5 19	2 12			2		
	pH	3		19	20	18 19 20		3	
	Nicotine	4		4		4	22	4	
	TSNA sum	5		3			3		
	Water after	9	18	6 15		15 17			9
Sample 9	Water before				18				
	Moisture		22	20	15	20	15		
	pH	3		4		4 18		3	
	Nicotine	4		1	19		1		4
	TSNA sum	5		3 15			3		
	Water after	9		17	6	6 17	2		

After the removal of outlying data, the mean, repeatability standard deviation (r SD), reproducibility standard deviation (R SD), repeatability (r) and reproducibility (R) for each sample and analyte yield were calculated from the remaining data. The statistical model used is given in ISO 5725-2.

The repeatability and reproducibility standard deviations together with the actual r and R figures for the nine smokeless tobacco products are listed in Tables 6-14.

Table 6: r&R figures for Sample 1.

Sample 1								
Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Water before	18,50	0,19	1,19	0,54	3,33	16	1,1%	6,4%
Moisture	24,08	0,34	0,74	0,95	2,09	19	1,4%	3,1%
pH	8,06	0,02	0,12	0,04	0,34	17	0,2%	1,5%
Nicotine	0,718	0,020	0,065	0,056	0,183	20	2,8%	9,1%
TSNA sum	2,344	0,067	0,248	0,186	0,695	16	2,8%	10,6%
Water after	18,28	0,20	1,04	0,55	2,92	17	1,1%	5,7%

Table 7: r&R figures for Sample 2.

Sample 2								
Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Water before	51,94	0,56	3,04	1,57	8,50	16	1,1%	5,8%
Moisture	58,42	0,31	0,84	0,88	2,34	20	0,5%	1,4%
pH	8,38	0,03	0,16	0,10	0,45	17	0,4%	1,9%
Nicotine	0,765	0,006	0,057	0,017	0,159	17	0,8%	7,4%
TSNA sum	0,581	0,016	0,107	0,046	0,299	15	2,8%	18,4%
Water after	51,47	0,72	2,78	2,02	7,79	18	1,4%	5,4%

Table 8: r&R figures for Sample 3.

Sample 3								
Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Water before	18,17	0,39	1,17	1,08	3,27	16	2,1%	6,4%
Moisture	22,16	0,29	1,81	0,80	5,06	20	1,3%	8,2%
pH	5,25	0,01	0,07	0,04	0,19	18	0,3%	1,3%
Nicotine	2,248	0,053	0,222	0,150	0,623	20	2,4%	9,9%
TSNA sum	0,924	0,067	0,204	0,188	0,570	19	7,3%	22,0%
Water after	17,60	0,33	1,88	0,93	5,27	18	1,9%	10,7%

Table 9: r&R figures for Sample 4.

Sample 4								
Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Water before	4,41	0,10	0,80	0,28	2,25	14	2,3%	18,2%
Moisture	5,44	0,11	0,62	0,31	1,73	20	2,1%	11,4%
pH	8,64	0,03	0,17	0,08	0,46	19	0,3%	1,9%
Nicotine	0,243	0,014	0,032	0,040	0,091	21	5,8%	13,4%
TSNA sum	0,781	0,032	0,174	0,089	0,486	16	4,1%	22,2%
Water after	4,15	0,15	0,75	0,41	2,10	17	3,6%	18,0%

Table 10: r&R figures for Sample 5.

Sample 5								
Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Water before	3,96	0,12	0,80	0,33	2,24	16	3,0%	20,1%
Moisture	4,84	0,11	0,40	0,31	1,12	20	2,3%	8,3%
pH	7,59	0,03	0,11	0,08	0,29	17	0,4%	1,4%
Nicotine	0,400	0,008	0,052	0,023	0,146	20	2,1%	13,0%
TSNA sum	0,635	0,022	0,133	0,062	0,371	15	3,5%	20,9%
Water after	4,07	0,13	0,55	0,36	1,54	18	3,2%	13,5%

Table 11: r&R figures for Sample 6.

Sample 6								
Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Water before	24,00	0,33	1,81	0,93	5,06	17	1,4%	7,5%
Moisture	26,29	0,25	1,31	0,69	3,65	20	0,9%	5,0%
pH	5,99	0,02	0,08	0,06	0,22	20	0,4%	1,3%
Nicotine	0,837	0,010	0,066	0,028	0,185	17	1,2%	7,9%
TSNA sum	0,886	0,029	0,136	0,081	0,380	16	3,3%	15,3%
Water after	23,62	0,30	1,94	0,85	5,42	18	1,3%	8,2%

Table 12: r&R figures for Sample 7.

Sample 7								
Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Water before	52,64	0,48	2,44	1,33	6,84	16	0,9%	4,6%
Moisture	55,43	0,12	0,49	0,33	1,38	20	0,2%	0,9%
pH	7,74	0,02	0,10	0,06	0,29	18	0,3%	1,4%
Nicotine	1,213	0,013	0,085	0,037	0,237	20	1,1%	7,0%
TSNA sum	5,214	0,126	0,742	0,352	2,076	19	2,4%	14,2%
Water after	51,19	0,40	4,32	1,11	12,09	16	0,8%	8,4%

Table 13: r&R figures for Sample 8.

Sample 8								
Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Water before	48,70	0,49	2,26	1,37	6,33	16	1,0%	4,6%
Moisture	52,66	0,24	0,63	0,68	1,77	20	0,5%	1,2%
pH	8,27	0,02	0,13	0,04	0,38	16	0,2%	1,6%
Nicotine	1,237	0,027	0,087	0,077	0,243	20	2,2%	7,0%
TSNA sum	6,472	0,189	0,932	0,530	2,609	19	2,9%	14,4%
Water after	47,69	0,29	3,76	0,81	10,53	15	0,6%	7,9%

Table 14: r&R figures for Sample 9.

Sample 9								
Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Water before	29,15	0,40	1,90	1,11	5,33	17	1,4%	6,5%
Moisture	32,20	0,24	0,93	0,68	2,61	19	0,8%	2,9%
pH	7,41	0,02	0,10	0,05	0,29	17	0,2%	1,4%
Nicotine	1,496	0,063	0,172	0,177	0,481	20	4,2%	11,5%
TSNA sum	1,543	0,056	0,251	0,158	0,703	19	3,6%	16,3%
Water after	28,75	0,46	2,59	1,28	7,25	16	1,6%	9,0%

For some analyte yields separate r&R figures were derived, depending on the analytical method applied. Prior to each of the calculations separate COCHRAN's and GRUBBS' tests were applied on the respective data. Outlying data was discarded.

r&R results for Water before and after per method:
Two methods were applied by the laboratories: GC and KF.

	Sample 1								
	Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
GC	**Water before**	17,75	0,15	0,80	0,41	2,24	6	0,8%	4,5%
	Water after	18,04	0,12	0,94	0,34	2,64	7	0,7%	5,2%
KF	**Water before**	18,94	0,22	1,19	0,61	3,33	10	1,1%	6,3%
	Water after	18,45	0,23	1,12	0,66	3,14	10	1,3%	6,1%

	Sample 2								
	Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
GC	**Water before**	51,90	0,54	2,01	1,50	5,62	5	1,0%	3,9%
	Water after	51,86	0,53	1,83	1,48	5,13	6	1,0%	3,5%
KF	**Water before**	52,76	0,60	2,40	1,68	6,73	10	1,1%	4,6%
	Water after	51,84	0,84	2,68	2,34	7,49	11	1,6%	5,2%

	Sample 3								
	Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
GC	**Water before**	17,43	0,15	2,85	0,43	7,98	5	0,9%	16,4%
	Water after	17,36	0,41	2,71	1,14	7,58	7	2,3%	15,6%
KF	**Water before**	17,76	0,41	0,95	1,15	2,66	11	2,3%	5,4%
	Water after	17,76	0,28	1,24	0,78	3,47	11	1,6%	7,0%

	Sample 4								
	Parameter	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
GC	**Water before**	4,95	0,03	0,73	0,09	2,04	5	0,6%	14,7%
	Water after	4,36	0,16	0,98	0,43	2,74	7	3,6%	22,5%
KF	**Water before**	4,15	0,29	0,74	0,81	2,07	11	7,0%	17,8%
	Water after	4,00	0,14	0,54	0,40	1,52	10	3,6%	13,6%

	Sample 5								
	Parameter	Mean	r SD	R SD	r	R	N	CV r	CV R
GC	Water before	4,10	0,06	0,57	0,17	1,60	6	1,5%	13,9%
	Water after	4,09	0,05	0,70	0,14	1,97	6	1,2%	17,2%
KF	Water before	3,89	0,14	0,93	0,40	2,60	10	3,7%	23,9%
	Water after	4,21	0,13	0,24	0,37	0,67	10	3,2%	5,7%

	Sample 6								
	Parameter	Mean	r SD	R SD	r	R	N	CV r	CV R
GC	Water before	23,42	0,26	2,34	0,72	6,57	6	1,1%	10,0%
	Water after	22,77	0,11	2,73	0,30	7,64	6	0,5%	12,0%
KF	Water before	24,31	0,37	1,47	1,03	4,11	11	1,5%	6,0%
	Water after	24,09	0,36	1,40	0,99	3,91	11	1,5%	5,8%

	Sample 7								
	Parameter	Mean	r SD	R SD	r	R	N	CV r	CV R
GC	Water before	49,79	0,40	5,49	1,11	15,38	6	0,8%	11,0%
	Water after	50,20	0,25	5,59	0,70	15,65	6	0,5%	11,1%
KF	Water before	53,02	0,50	2,38	1,41	6,66	11	0,9%	4,5%
	Water after	52,04	0,37	3,66	1,04	10,25	9	0,7%	7,0%

	Sample 8								
	Parameter	Mean	r SD	R SD	r	R	N	CV r	CV R
GC	Water before	46,28	0,79	3,80	2,22	10,63	6	1,7%	8,2%
	Water after	46,52	0,24	4,41	0,66	12,35	6	0,5%	9,5%
KF	Water before	49,14	0,46	2,53	1,30	7,08	11	0,9%	5,1%
	Water after	48,47	0,32	3,30	0,90	9,24	9	0,7%	6,8%

	Sample 9								
	Parameter	Mean	r SD	R SD	r	R	N	CV r	CV R
GC	Water before	27,68	0,28	1,33	0,79	3,73	6	1,0%	4,8%
	Water after	27,49	0,25	2,52	0,71	7,04	7	0,9%	9,2%
KF	Water before	29,95	0,44	1,70	1,24	4,76	11	1,5%	5,7%
	Water after	29,53	0,69	2,30	1,95	6,44	10	2,4%	7,8%

r&R results for Nicotine per method:

Three different method groups were considered: CRM62, CDC and OWN.

Sample 1								
Method	Mean	r SD	R SD	r	R	N	CV r	CV R
CRM62	0,707	0,018	0,064	0,050	0,180	4	2,5%	9,1%
CDC	0,707	0,008	0,036	0,022	0,101	6	1,1%	5,1%
OWN	0,746	0,026	0,081	0,073	0,228	8	3,5%	10,9%

Sample 2								
Method	Mean	r SD	R SD	r	R	N	CV r	CV R
CRM62	0,757	0,005	0,070	0,014	0,195	4	0,7%	9,2%
CDC	0,751	0,007	0,038	0,019	0,105	6	0,9%	5,0%
OWN	0,793	0,024	0,059	0,068	0,166	8	3,0%	7,5%

Sample 3								
Method	Mean	r SD	R SD	r	R	N	CV r	CV R
CRM62	2,293	0,015	0,119	0,043	0,334	3	0,7%	5,2%
CDC	2,291	0,048	0,123	0,135	0,345	6	2,1%	5,4%
OWN	2,319	0,051	0,255	0,144	0,713	8	2,2%	11,0%

Sample 4								
Method	Mean	r SD	R SD	r	R	N	CV r	CV R
CRM62	0,258	0,011	0,041	0,031	0,114	4	4,3%	15,8%
CDC	0,247	0,013	0,042	0,038	0,117	7	5,5%	16,9%
OWN	0,233	0,015	0,020	0,043	0,056	8	6,6%	8,7%

Sample 5								
Method	Mean	r SD	R SD	r	R	N	CV r	CV R
CRM62	0,387	0,005	0,051	0,013	0,144	4	1,2%	13,3%
CDC	0,399	0,008	0,050	0,023	0,140	7	2,0%	12,5%
OWN	0,410	0,010	0,062	0,028	0,174	8	2,5%	15,2%

Sample 6								
Method	Mean	r SD	R SD	r	R	N	CV r	CV R
CRM62	0,833	0,019	0,084	0,053	0,234	4	2,3%	10,0%
CDC	0,840	0,010	0,043	0,027	0,122	6	1,1%	5,2%
OWN	0,855	0,011	0,090	0,031	0,253	6	1,3%	10,6%

Sample 7								
Method	Mean	r SD	R SD	r	R	N	CV r	CV R
CRM62	1,169	0,015	0,088	0,043	0,247	4	1,3%	7,5%
CDC	1,196	0,013	0,051	0,037	0,144	6	1,1%	4,3%
OWN	1,260	0,013	0,098	0,036	0,273	8	1,0%	7,7%

Sample 8								
Method	Mean	r SD	R SD	r	R	N	CV r	CV R
CRM62	1,187	0,024	0,076	0,068	0,212	4	2,0%	6,4%
CDC	1,237	0,019	0,060	0,054	0,167	6	1,6%	4,8%
OWN	1,274	0,035	0,107	0,098	0,300	8	2,8%	8,4%

Sample 9								
Method	Mean	r SD	R SD	r	R	N	CV r	CV R
CRM62	1,452	0,037	0,106	0,104	0,297	3	2,6%	7,3%
CDC	1,523	0,021	0,131	0,058	0,366	5	1,3%	8,6%
OWN	1,576	0,068	0,141	0,189	0,394	8	4,3%	8,9%

r&R results for pH per method:

Two method groups were distinguished: CDC and CDC?. The results refer to pH measurements after 30 minutes extraction time.

Method	CDC							
	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	8,07	0,018	0,131	0,050	0,367	11	0,2%	1,6%
Sample 2	8,42	0,038	0,144	0,105	0,404	11	0,4%	1,7%
Sample 3	5,27	0,014	0,076	0,039	0,214	11	0,3%	1,4%
Sample 4	8,65	0,021	0,130	0,059	0,364	11	0,2%	1,5%
Sample 5	7,61	0,029	0,108	0,080	0,302	12	0,4%	1,4%
Sample 6	6,00	0,012	0,088	0,033	0,247	11	0,2%	1,5%
Sample 7	7,77	0,034	0,112	0,095	0,314	12	0,4%	1,4%
Sample 8	8,30	0,016	0,149	0,046	0,417	10	0,2%	1,8%
Sample 9	7,41	0,020	0,123	0,057	0,344	10	0,3%	1,7%

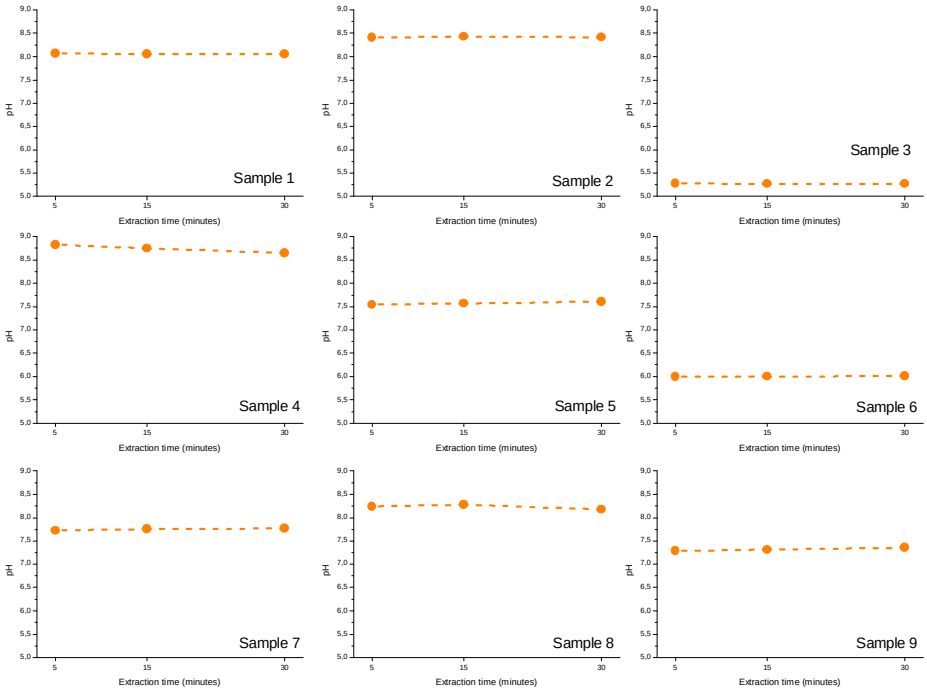
Method	CDC?							
	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	8,04	0,010	0,105	0,027	0,294	6	0,1%	1,3%
Sample 2	8,19	0,093	0,366	0,259	1,024	8	1,1%	4,5%
Sample 3	5,22	0,015	0,050	0,041	0,139	7	0,3%	1,0%
Sample 4	8,62	0,038	0,214	0,105	0,600	8	0,4%	2,5%
Sample 5	7,63	0,071	0,206	0,199	0,576	8	0,9%	2,7%
Sample 6	5,97	0,029	0,067	0,081	0,187	8	0,5%	1,1%
Sample 7	7,68	0,015	0,060	0,041	0,168	7	0,2%	0,8%
Sample 8	8,21	0,041	0,093	0,115	0,261	8	0,5%	1,1%
Sample 9	7,42	0,012	0,071	0,033	0,200	7	0,2%	1,0%

The pH of the nine samples was measured after 5, 15 and 30 minutes extraction time.

The following graph shows the change in pH depending on the extraction time. As the y-axis for all nine plots is chosen the same, the different pH levels of the products can be seen easily.

It seems as if a longer extraction time has no major effect on the pH measurement.

Change in pH depending on extraction time



Plot: pH depending on extraction time.

r&R results for TSNAs:

As mentioned in the introduction to this report, 15 laboratories reported results for a TSNA standard that was sent out to the participating laboratories. The results were used to correct the reported TSNA results for the difference between the laboratories' calibration standards. Therefore, for TSNAs two results were calculated, "*TSNAs as received*"" and "*TSNAs corrected*". The correction factors are listed below the r&R Tables for the TSNAs.

TSNA sum as received								
	Mean	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Sample 1	2,344	0,067	0,248	0,186	0,695	16	2,8%	10,6%
Sample 2	0,581	0,016	0,107	0,046	0,299	15	2,8%	18,4%
Sample 3	0,924	0,067	0,204	0,188	0,570	19	7,3%	22,0%
Sample 4	0,781	0,032	0,174	0,089	0,486	16	4,1%	22,2%
Sample 5	0,635	0,022	0,133	0,062	0,371	15	3,5%	20,9%
Sample 6	0,886	0,029	0,136	0,081	0,380	16	3,3%	15,3%
Sample 7	5,214	0,126	0,742	0,352	2,076	19	2,4%	14,2%
Sample 8	6,472	0,189	0,932	0,530	2,609	19	2,9%	14,4%
Sample 9	1,543	0,056	0,251	0,158	0,703	19	3,6%	16,3%

NNN as received

	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	1,110	0,038	0,181	0,107	0,508	17	3,4%	16,3%
Sample 2	0,262	0,015	0,049	0,043	0,138	17	5,8%	18,8%
Sample 3	0,538	0,039	0,115	0,110	0,322	19	7,3%	21,4%
Sample 4	0,463	0,022	0,082	0,062	0,229	18	4,8%	17,6%
Sample 5	0,218	0,017	0,059	0,049	0,165	18	8,0%	26,9%
Sample 6	0,503	0,019	0,081	0,053	0,227	17	3,8%	16,2%
Sample 7	2,194	0,043	0,362	0,121	1,014	18	2,0%	16,5%
Sample 8	2,515	0,057	0,382	0,160	1,068	17	2,3%	15,2%
Sample 9	0,711	0,034	0,116	0,094	0,326	19	4,7%	16,4%

NAT as received

	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,668	0,026	0,130	0,074	0,363	18	3,9%	19,4%
Sample 2	0,176	0,014	0,040	0,038	0,113	16	7,7%	22,9%
Sample 3	0,313	0,029	0,097	0,080	0,271	19	9,1%	30,9%
Sample 4	0,133	0,008	0,050	0,021	0,140	16	5,7%	37,6%
Sample 5	0,185	0,018	0,058	0,050	0,163	18	9,6%	31,4%
Sample 6	0,290	0,023	0,072	0,064	0,202	18	7,9%	24,9%
Sample 7	2,161	0,085	0,412	0,239	1,155	19	4,0%	19,1%
Sample 8	3,204	0,095	0,657	0,267	1,841	18	3,0%	20,5%
Sample 9	0,545	0,026	0,114	0,073	0,320	18	4,8%	20,9%

NAB as received

	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,041	0,003	0,009	0,007	0,025	11	6,2%	21,5%
Sample 2	0,015	0,001	0,003	0,003	0,008	11	6,6%	19,0%
Sample 3	0,016	0,002	0,003	0,005	0,008	10	11,0%	17,9%
Sample 4	0,051	0,003	0,009	0,007	0,026	13	5,3%	18,6%
Sample 5	0,021	0,002	0,007	0,006	0,019	12	9,9%	32,1%
Sample 6	0,014	0,001	0,002	0,002	0,006	9	6,1%	15,9%
Sample 7	0,182	0,008	0,030	0,023	0,083	15	4,6%	16,2%
Sample 8	0,208	0,007	0,048	0,020	0,134	14	3,5%	23,1%
Sample 9	0,045	0,003	0,011	0,007	0,030	13	5,8%	23,7%

NNK as received

	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,501	0,027	0,067	0,074	0,187	18	5,3%	13,3%
Sample 2	0,131	0,006	0,019	0,016	0,054	13	4,2%	14,7%
Sample 3	0,079	0,011	0,014	0,030	0,039	14	13,5%	17,4%
Sample 4	0,153	0,010	0,020	0,028	0,057	13	6,4%	13,3%
Sample 5	0,232	0,021	0,046	0,058	0,128	16	8,9%	19,7%
Sample 6	0,097	0,009	0,013	0,025	0,037	14	9,4%	13,7%
Sample 7	0,685	0,021	0,069	0,059	0,193	17	3,1%	10,1%
Sample 8	0,556	0,021	0,050	0,058	0,141	17	3,8%	9,0%
Sample 9	0,271	0,011	0,032	0,030	0,089	18	4,0%	11,8%

TSNA sum corrected

	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	2,365	0,065	0,393	0,183	1,101	13	2,8%	16,6%
Sample 2	0,621	0,011	0,096	0,032	0,270	11	1,8%	15,5%
Sample 3	0,947	0,068	0,235	0,190	0,659	15	7,2%	24,9%
Sample 4	0,838	0,035	0,176	0,098	0,494	14	4,2%	21,1%
Sample 5	0,668	0,040	0,156	0,113	0,436	14	6,1%	23,3%
Sample 6	0,936	0,037	0,131	0,105	0,367	13	4,0%	14,0%
Sample 7	5,432	0,081	0,933	0,227	2,612	14	1,5%	17,2%
Sample 8	6,751	0,200	1,060	0,560	2,968	15	3,0%	15,7%
Sample 9	1,622	0,038	0,284	0,106	0,794	14	2,3%	17,5%

NNN corrected

	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	1,149	0,071	0,190	0,199	0,533	15	6,2%	16,6%
Sample 2	0,278	0,014	0,050	0,040	0,140	13	5,2%	17,9%
Sample 3	0,544	0,038	0,118	0,106	0,330	15	6,9%	21,6%
Sample 4	0,480	0,024	0,074	0,066	0,208	14	4,9%	15,5%
Sample 5	0,231	0,018	0,063	0,049	0,177	14	7,6%	27,3%
Sample 6	0,521	0,021	0,066	0,059	0,185	13	4,0%	12,7%
Sample 7	2,283	0,044	0,454	0,122	1,271	15	1,9%	19,9%
Sample 8	2,575	0,063	0,411	0,176	1,150	13	2,4%	15,9%
Sample 9	0,733	0,035	0,123	0,098	0,345	15	4,8%	16,8%

NAT corrected

	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,685	0,027	0,095	0,076	0,265	14	4,0%	13,8%
Sample 2	0,185	0,012	0,034	0,034	0,096	13	6,5%	18,6%
Sample 3	0,313	0,028	0,091	0,077	0,255	15	8,8%	29,1%
Sample 4	0,134	0,007	0,036	0,021	0,100	14	5,5%	26,7%
Sample 5	0,192	0,017	0,052	0,047	0,145	14	8,7%	26,9%
Sample 6	0,289	0,021	0,058	0,060	0,163	14	7,4%	20,1%
Sample 7	2,224	0,090	0,325	0,251	0,910	15	4,0%	14,6%
Sample 8	3,358	0,090	0,468	0,252	1,312	14	2,7%	14,0%
Sample 9	0,558	0,024	0,081	0,068	0,226	14	4,3%	14,5%

NAB corrected

	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,042	0,003	0,012	0,007	0,033	11	6,3%	28,2%
Sample 2	0,015	0,001	0,004	0,003	0,011	11	6,5%	25,3%
Sample 3	0,016	0,002	0,004	0,005	0,011	10	11,5%	24,4%
Sample 4	0,051	0,003	0,012	0,008	0,033	12	5,6%	23,4%
Sample 5	0,021	0,002	0,009	0,006	0,024	11	10,5%	39,8%
Sample 6	0,014	0,001	0,003	0,002	0,009	9	6,0%	22,7%
Sample 7	0,181	0,008	0,034	0,023	0,094	13	4,5%	18,5%
Sample 8	0,203	0,007	0,043	0,019	0,120	12	3,3%	21,2%
Sample 9	0,044	0,003	0,013	0,008	0,037	12	6,5%	29,5%

NNK corrected								
	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,484	0,030	0,082	0,084	0,228	14	6,2%	16,8%
Sample 2	0,133	0,006	0,023	0,016	0,064	12	4,3%	17,1%
Sample 3	0,081	0,012	0,019	0,034	0,053	13	15,0%	23,3%
Sample 4	0,154	0,011	0,027	0,030	0,077	12	7,0%	17,8%
Sample 5	0,236	0,021	0,059	0,059	0,166	13	8,9%	25,2%
Sample 6	0,099	0,010	0,023	0,029	0,065	13	10,4%	23,3%
Sample 7	0,734	0,019	0,155	0,054	0,434	15	2,6%	21,1%
Sample 8	0,570	0,022	0,088	0,061	0,246	14	3,8%	15,4%
Sample 9	0,283	0,010	0,071	0,029	0,197	15	3,6%	24,9%

The following Table lists the correction factors derived from TSNA standards.

Table: Correction factors from derived from TSNA standards.					
LAB Code	TSNA sum	NNN	NAT	NAB	NNK
1	1,0780	1,0190	1,0757	1,1834	1,0682
2	1,0081	0,9852	1,0050	0,9971	0,9985
3	1,0203	0,9475	1,0118	1,1065	1,0440
4	1,1287	1,1225	0,9449	1,0165	1,3788
5					
6	0,8162	0,7679	1,0317		0,8053
7	0,9901	0,9192	1,0367	0,8958	0,9957
8	0,9990	0,9784	1,0248	0,9559	0,9576
9	0,8889	0,9071	0,8580	0,9125	0,8637
10	1,2145	1,2333	1,1995	1,1008	1,1974
11					
12	0,9115	0,9160	0,8705	0,8543	0,9421
13	0,9003	0,9165	0,8513	0,9089	0,9072
14	0,7873	1,0120	0,4614	0,9084	0,9041
15					
16					
17	0,9556	0,8657	1,0091	0,9336	0,9517
18					
19					
20					
21	0,9494	1,1077	1,2759		0,5812
22					
23	1,3521	1,3019	1,3436	1,2264	1,4046

The following plot shows the raw data for TSNA sum corrected via TSNA standards.

Separate results for three methods were derived. The methods applied by the laboratories were LCMSMS SMNE, LCMSMS and GC.
The following Tables list the r&R figures for TSNAs (as received and corrected) per method applied.

TSNAs as received:

TSNA sum as received								
LCMSMS SMNE	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	2,250	0,036	0,178	0,102	0,498	8	1,6%	7,9%
Sample 2	0,606	0,010	0,051	0,028	0,144	8	1,6%	8,5%
Sample 3	0,943	0,062	0,204	0,174	0,570	9	6,6%	21,6%
Sample 4	0,803	0,036	0,082	0,100	0,229	8	4,5%	10,2%
Sample 5	0,663	0,016	0,053	0,044	0,148	6	2,4%	8,0%
Sample 6	0,906	0,029	0,067	0,080	0,188	8	3,2%	7,4%
Sample 7	5,161	0,084	0,597	0,234	1,673	9	1,6%	11,6%
Sample 8	6,473	0,219	0,813	0,613	2,276	9	3,4%	12,6%
Sample 9	1,521	0,060	0,202	0,167	0,565	9	3,9%	13,3%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	2,434	0,080	0,262	0,224	0,733	3	3,3%	10,8%
Sample 2	0,574	0,037	0,124	0,104	0,348	3	6,4%	21,7%
Sample 3	0,877	0,074	0,076	0,206	0,213	3	8,4%	8,7%
Sample 4	0,788	0,036	0,143	0,101	0,401	3	4,6%	18,2%
Sample 5	0,712					2		
Sample 6	0,809	0,141	0,242	0,394	0,678	3	17,4%	29,9%
Sample 7	5,475	0,177	0,915	0,496	2,562	3	3,2%	16,7%
Sample 8	6,958	0,186	1,127	0,520	3,156	3	2,7%	16,2%
Sample 9	1,686	0,043	0,273	0,121	0,765	3	2,6%	16,2%

GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	2,237	0,083	0,575	0,233	1,611	6	3,7%	25,7%
Sample 2	0,518	0,023	0,163	0,064	0,458	5	4,4%	31,5%
Sample 3	0,919	0,071	0,253	0,198	0,710	7	7,7%	27,6%
Sample 4	0,743	0,020	0,296	0,057	0,830	5	2,7%	39,9%
Sample 5	0,588	0,028	0,157	0,079	0,440	7	4,8%	26,7%
Sample 6	0,867	0,026	0,199	0,074	0,556	7	3,0%	22,9%
Sample 7	5,170	0,144	0,921	0,402	2,579	7	2,8%	17,8%
Sample 8	6,262	0,144	1,059	0,403	2,965	7	2,3%	16,9%
Sample 9	1,527	0,032	0,334	0,089	0,935	6	2,1%	21,9%

NNN as received								
LCMSMS SMNE	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	1,089	0,025	0,133	0,069	0,372	8	2,3%	12,2%
Sample 2	0,276	0,008	0,028	0,021	0,078	8	2,7%	10,1%
Sample 3	0,555	0,033	0,091	0,093	0,256	9	6,0%	16,5%
Sample 4	0,480	0,023	0,059	0,064	0,164	8	4,8%	12,2%
Sample 5	0,213	0,017	0,049	0,047	0,137	8	7,9%	22,9%
Sample 6	0,511	0,019	0,039	0,052	0,109	8	3,6%	7,6%
Sample 7	2,171	0,033	0,369	0,094	1,034	9	1,5%	17,0%
Sample 8	2,496	0,063	0,261	0,175	0,730	8	2,5%	10,5%
Sample 9	0,694	0,039	0,093	0,108	0,259	9	5,6%	13,3%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	1,208	0,054	0,143	0,151	0,400	3	4,5%	11,8%
Sample 2	0,253	0,021	0,069	0,059	0,194	3	8,3%	27,5%
Sample 3	0,476	0,036	0,046	0,102	0,128	3	7,6%	9,6%
Sample 4	0,461	0,022	0,099	0,063	0,276	3	4,8%	21,4%
Sample 5	0,243	0,029	0,093	0,080	0,260	3	11,8%	38,2%
Sample 6	0,451	0,092	0,138	0,258	0,387	3	20,4%	30,6%
Sample 7	2,367	0,069	0,399	0,192	1,118	3	2,9%	16,9%
Sample 8	2,711	0,124	0,523	0,349	1,464	3	4,6%	19,3%
Sample 9	0,774	0,033	0,150	0,091	0,420	3	4,2%	19,4%

GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	1,090	0,043	0,254	0,120	0,712	6	3,9%	23,3%
Sample 2	0,248	0,019	0,064	0,053	0,178	6	7,6%	25,7%
Sample 3	0,543	0,047	0,159	0,131	0,444	7	8,6%	29,2%
Sample 4	0,446	0,021	0,104	0,060	0,291	7	4,8%	23,3%
Sample 5	0,214	0,010	0,060	0,029	0,169	7	4,8%	28,3%
Sample 6	0,492	0,011	0,120	0,030	0,337	7	2,2%	24,5%
Sample 7	2,141	0,039	0,375	0,110	1,051	6	1,8%	17,5%
Sample 8	2,447	0,053	0,443	0,149	1,239	7	2,2%	18,1%
Sample 9	0,705	0,026	0,138	0,073	0,386	7	3,7%	19,5%

NAT as received								
LCMSMS SMNE	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Sample 1	0,647	0,024	0,133	0,068	0,373	9	3,7%	20,6%
Sample 2	0,176	0,004	0,044	0,012	0,122	7	2,3%	24,7%
Sample 3	0,305	0,027	0,111	0,075	0,311	9	8,7%	36,4%
Sample 4	0,123	0,007	0,033	0,019	0,091	8	5,6%	26,6%
Sample 5	0,171	0,016	0,051	0,045	0,142	8	9,3%	29,6%
Sample 6	0,287	0,006	0,065	0,017	0,182	7	2,1%	22,6%
Sample 7	2,091	0,062	0,461	0,174	1,290	9	3,0%	22,0%
Sample 8	3,151	0,134	0,694	0,376	1,943	9	4,3%	22,0%
Sample 9	0,529	0,024	0,128	0,067	0,358	9	4,5%	24,2%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,692	0,036	0,085	0,099	0,238	3	5,1%	12,3%
Sample 2	0,170	0,015	0,036	0,042	0,100	3	8,7%	21,0%
Sample 3	0,299	0,024	0,031	0,066	0,087	3	7,9%	10,3%
Sample 4	0,126	0,008	0,018	0,024	0,051	3	6,7%	14,5%
Sample 5	0,171	0,027	0,033	0,075	0,092	3	15,6%	19,3%
Sample 6	0,246	0,042	0,090	0,119	0,253	3	17,2%	36,6%
Sample 7	2,216	0,153	0,407	0,428	1,140	3	6,9%	18,4%
Sample 8	3,445	0,103	0,540	0,288	1,512	3	3,0%	15,7%
Sample 9	0,576	0,026	0,103	0,072	0,289	3	4,5%	17,9%

GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,688	0,024	0,155	0,067	0,434	6	3,5%	22,5%
Sample 2	0,171	0,019	0,044	0,053	0,123	5	11,1%	25,7%
Sample 3	0,328	0,033	0,103	0,092	0,289	7	10,0%	31,4%
Sample 4	0,154	0,008	0,072	0,021	0,202	6	4,9%	46,9%
Sample 5	0,207	0,008	0,078	0,021	0,219	6	3,7%	37,8%
Sample 6	0,311	0,019	0,077	0,054	0,216	7	6,2%	24,8%
Sample 7	2,226	0,069	0,394	0,195	1,102	7	3,1%	17,7%
Sample 8	3,165	0,088	0,677	0,247	1,896	7	2,8%	21,4%
Sample 9	0,553	0,029	0,112	0,082	0,313	6	5,3%	20,2%

NAB as received

LCMSMS SMNE	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,039	0,002	0,005	0,006	0,013	8	5,4%	12,1%
Sample 2	0,015	0,001	0,002	0,003	0,006	8	6,7%	15,4%
Sample 3	0,016	0,002	0,003	0,005	0,008	8	11,3%	18,0%
Sample 4	0,048	0,002	0,008	0,007	0,022	8	5,0%	16,0%
Sample 5	0,019	0,002	0,005	0,005	0,013	8	8,8%	25,1%
Sample 6	0,014	0,001	0,002	0,002	0,006	8	6,3%	16,1%
Sample 7	0,167	0,006	0,024	0,015	0,068	8	3,3%	14,5%
Sample 8	0,189	0,005	0,036	0,015	0,100	8	2,8%	18,9%
Sample 9	0,038	0,002	0,005	0,005	0,015	8	5,1%	13,9%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,047	0,004	0,015	0,010	0,043	3	7,5%	32,5%
Sample 2	0,022	0,002	0,015	0,006	0,043	3	10,5%	69,9%
Sample 3	0,026					2		
Sample 4	0,051	0,003	0,014	0,008	0,040	3	5,6%	27,8%
Sample 5	0,026	0,003	0,011	0,009	0,030	3	12,0%	40,5%
Sample 6	0,025					2		
Sample 7	0,200	0,010	0,014	0,027	0,039	3	4,8%	7,0%
Sample 8	0,222	0,010	0,012	0,028	0,035	3	4,5%	5,6%
Sample 9	0,054	0,003	0,010	0,010	0,029	3	6,4%	19,3%

GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,068					2		
Sample 2	0,018					2		
Sample 3	0,016					2		
Sample 4	0,059					2		
Sample 5	0,025					2		
Sample 6	0,021					2		
Sample 7	0,202	0,007	0,040	0,018	0,113	3	3,2%	20,0%
Sample 8	0,244	0,008	0,081	0,023	0,226	3	3,4%	33,1%
Sample 9	0,056					2		

NNK as received								
LCMSMS SMNE	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,482	0,016	0,047	0,046	0,131	9	3,4%	9,7%
Sample 2	0,133	0,005	0,015	0,014	0,043	8	3,8%	11,6%
Sample 3	0,078	0,006	0,011	0,016	0,031	8	7,1%	14,2%
Sample 4	0,152	0,009	0,016	0,026	0,044	8	6,1%	10,3%
Sample 5	0,246	0,011	0,026	0,032	0,071	7	4,6%	10,4%
Sample 6	0,094	0,005	0,009	0,014	0,025	8	5,2%	9,5%
Sample 7	0,729	0,020	0,180	0,056	0,505	9	2,8%	24,7%
Sample 8	0,583	0,027	0,133	0,075	0,372	9	4,6%	22,8%
Sample 9	0,265	0,005	0,026	0,014	0,073	8	1,8%	9,9%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,486	0,035	0,078	0,099	0,218	3	7,3%	16,0%
Sample 2	0,129	0,007	0,025	0,021	0,071	3	5,8%	19,6%
Sample 3	0,085	0,016	0,016	0,045	0,046	3	19,2%	19,5%
Sample 4	0,150	0,013	0,032	0,038	0,090	3	9,0%	21,5%
Sample 5	0,232	0,024	0,070	0,066	0,196	3	10,2%	30,1%
Sample 6	0,095	0,015	0,015	0,041	0,041	3	15,2%	15,4%
Sample 7	0,692	0,025	0,119	0,071	0,332	3	3,6%	17,1%
Sample 8	0,579	0,016	0,087	0,044	0,244	3	2,7%	15,1%
Sample 9	0,282	0,014	0,041	0,039	0,116	3	5,0%	14,6%

GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,538	0,033	0,081	0,092	0,226	6	6,1%	15,0%
Sample 2	0,131	0,011	0,030	0,032	0,084	3	8,8%	23,1%
Sample 3	0,077	0,014	0,018	0,038	0,051	3	17,8%	23,6%
Sample 4	0,162					2		
Sample 5	0,230	0,022	0,037	0,060	0,102	5	9,4%	15,9%
Sample 6	0,105	0,011	0,019	0,030	0,055	3	10,1%	18,5%
Sample 7	0,698	0,024	0,066	0,068	0,186	6	3,5%	9,5%
Sample 8	0,565	0,024	0,039	0,067	0,110	6	4,2%	6,9%
Sample 9	0,273	0,013	0,040	0,036	0,111	6	4,7%	14,5%

TSNAs corrected:

TSNA sum corrected								
LCMSMS SMNE	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	2,247	0,036	0,398	0,101	1,116	8	1,6%	17,7%
Sample 2	0,612	0,010	0,105	0,029	0,295	8	1,7%	17,2%
Sample 3	0,947	0,063	0,267	0,176	0,748	9	6,6%	28,2%
Sample 4	0,815	0,037	0,167	0,103	0,468	8	4,5%	20,5%
Sample 5	0,652	0,041	0,174	0,114	0,487	8	6,2%	26,7%
Sample 6	0,914	0,029	0,142	0,080	0,396	8	3,1%	15,5%
Sample 7	5,145	0,085	0,958	0,237	2,682	9	1,6%	18,6%
Sample 8	6,439	0,221	1,194	0,618	3,343	9	3,4%	18,5%
Sample 9	1,521	0,040	0,326	0,112	0,912	8	2,6%	21,4%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	2,372	0,081	0,239	0,228	0,668	3	3,4%	10,1%
Sample 2	0,560	0,037	0,126	0,104	0,352	3	6,6%	22,5%
Sample 3	0,865	0,069	0,174	0,193	0,489	3	8,0%	20,2%
Sample 4	0,761	0,036	0,053	0,102	0,148	3	4,8%	6,9%
Sample 5	0,661	0,043	0,199	0,121	0,558	3	6,5%	30,2%
Sample 6	0,787	0,138	0,230	0,385	0,644	3	17,5%	29,2%
Sample 7	5,291	0,193	0,204	0,539	0,572	3	3,6%	3,9%
Sample 8	6,729	0,186	0,168	0,521	0,470	3	2,8%	2,5%
Sample 9	1,631	0,045	0,053	0,126	0,149	3	2,8%	3,3%

GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	2,819	0,282	0,316	0,790	0,885	3	10,0%	11,2%
Sample 2	0,693					2		
Sample 3	1,030	0,081	0,217	0,225	0,609	3	7,8%	21,1%
Sample 4	0,977	0,027	0,247	0,076	0,692	3	2,8%	25,3%
Sample 5	0,717	0,037	0,076	0,105	0,212	3	5,2%	10,6%
Sample 6	1,025	0,031	0,084	0,088	0,236	3	3,0%	8,2%
Sample 7	6,353	0,052	0,566	0,147	1,586	3	0,8%	8,9%
Sample 8	7,708	0,140	0,543	0,393	1,521	3	1,8%	7,0%
Sample 9	1,885	0,020	0,060	0,056	0,168	3	1,1%	3,2%

NNN corrected								
LCMSMS SMNE	**Mean**	**r SD**	**R SD**	**r**	**R**	**N**	**CV r**	**CV R**
Sample 1	1,093	0,025	0,223	0,071	0,625	8	2,3%	20,4%
Sample 2	0,282	0,008	0,045	0,022	0,127	8	2,8%	16,1%
Sample 3	0,562	0,035	0,134	0,097	0,377	9	6,2%	23,9%
Sample 4	0,490	0,024	0,092	0,068	0,257	8	4,9%	18,7%
Sample 5	0,221	0,016	0,061	0,044	0,170	8	7,2%	27,5%
Sample 6	0,521	0,019	0,078	0,054	0,220	8	3,7%	15,1%
Sample 7	2,203	0,034	0,541	0,096	1,514	9	1,5%	24,5%
Sample 8	2,585	0,105	0,537	0,295	1,502	9	4,1%	20,8%
Sample 9	0,701	0,039	0,145	0,110	0,407	9	5,6%	20,8%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	1,173	0,056	0,145	0,158	0,406	3	4,8%	12,4%
Sample 2	0,245	0,021	0,066	0,059	0,184	3	8,6%	26,8%
Sample 3	0,465	0,033	0,078	0,094	0,218	3	7,2%	16,7%
Sample 4	0,441	0,023	0,028	0,064	0,078	3	5,2%	6,3%
Sample 5	0,239	0,027	0,102	0,075	0,287	3	11,3%	42,9%
Sample 6	0,434	0,093	0,119	0,261	0,333	3	21,5%	27,3%
Sample 7	2,277	0,072	0,102	0,201	0,285	3	3,1%	4,5%
Sample 8	2,602	0,125	0,131	0,350	0,367	3	4,8%	5,0%
Sample 9	0,743	0,033	0,035	0,093	0,098	3	4,5%	4,7%

GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	1,242	0,102	0,107	0,287	0,300	3	8,2%	8,6%
Sample 2	0,357	0,065	0,095	0,183	0,267	3	18,3%	26,7%
Sample 3	0,571	0,049	0,075	0,137	0,209	3	8,6%	13,1%
Sample 4	0,489	0,023	0,050	0,064	0,139	3	4,7%	10,2%
Sample 5	0,252	0,007	0,032	0,020	0,091	3	2,9%	12,8%
Sample 6	0,547	0,013	0,015	0,037	0,043	3	2,4%	2,8%
Sample 7	2,339					2	0,0%	0,0%
Sample 8	2,744	0,072	0,291	0,200	0,815	3	2,6%	10,6%
Sample 9	0,818	0,020	0,063	0,056	0,178	3	2,4%	7,8%

NAT corrected

LCMSMS SMNE	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,680	0,025	0,109	0,069	0,304	9	3,6%	16,0%
Sample 2	0,186	0,004	0,034	0,012	0,096	7	2,3%	18,4%
Sample 3	0,320	0,027	0,107	0,076	0,299	9	8,5%	33,4%
Sample 4	0,128	0,007	0,027	0,019	0,075	8	5,2%	20,8%
Sample 5	0,182	0,015	0,051	0,041	0,143	8	8,1%	27,9%
Sample 6	0,307	0,006	0,047	0,018	0,133	7	2,1%	15,5%
Sample 7	2,195	0,066	0,374	0,186	1,048	9	3,0%	17,1%
Sample 8	3,306	0,134	0,572	0,375	1,600	9	4,0%	17,3%
Sample 9	0,553	0,024	0,100	0,067	0,280	9	4,3%	18,1%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,681	0,035	0,094	0,097	0,263	3	5,1%	13,8%
Sample 2	0,168	0,015	0,043	0,042	0,119	3	8,8%	25,4%
Sample 3	0,299	0,022	0,070	0,061	0,195	3	7,2%	23,3%
Sample 4	0,124	0,008	0,020	0,023	0,057	3	6,6%	16,3%
Sample 5	0,170	0,025	0,039	0,070	0,109	3	14,8%	22,9%
Sample 6	0,242	0,040	0,091	0,113	0,254	3	16,6%	37,4%
Sample 7	2,155	0,163	0,147	0,456	0,411	3	7,6%	6,8%
Sample 8	3,358	0,097	0,100	0,272	0,281	3	2,9%	3,0%
Sample 9	0,560	0,028	0,030	0,078	0,084	3	4,9%	5,4%

GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,712					2		
Sample 2	0,237	0,052	0,097	0,146	0,272	3	22,1%	41,0%
Sample 3	0,307	0,034	0,081	0,095	0,228	3	11,0%	26,5%
Sample 4	0,160	0,008	0,064	0,023	0,179	3	5,1%	40,0%
Sample 5	0,240	0,010	0,044	0,028	0,124	3	4,2%	18,4%
Sample 6	0,303	0,011	0,036	0,031	0,101	3	3,7%	11,9%
Sample 7	2,379	0,023	0,318	0,065	0,890	3	1,0%	13,4%
Sample 8	3,440	0,050	0,369	0,141	1,032	3	1,5%	10,7%
Sample 9	0,574					2		

NAB corrected								
LCMSMS SMNE	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,039	0,002	0,008	0,006	0,023	8	5,2%	20,6%
Sample 2	0,015	0,001	0,003	0,003	0,009	8	6,4%	22,5%
Sample 3	0,016	0,002	0,004	0,005	0,011	8	11,7%	24,0%
Sample 4	0,048	0,002	0,009	0,007	0,026	8	5,0%	19,4%
Sample 5	0,019	0,002	0,006	0,005	0,016	8	8,8%	30,5%
Sample 6	0,014	0,001	0,003	0,002	0,009	8	6,1%	22,5%
Sample 7	0,168	0,006	0,035	0,015	0,097	8	3,3%	20,5%
Sample 8	0,190	0,005	0,047	0,015	0,132	8	2,9%	24,7%
Sample 9	0,038	0,002	0,008	0,006	0,023	8	5,2%	21,5%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,049	0,004	0,019	0,010	0,053	3	7,7%	39,1%
Sample 2	0,023	0,002	0,017	0,007	0,049	3	11,0%	77,1%
Sample 3	0,027					2		
Sample 4	0,052	0,003	0,017	0,009	0,048	3	5,9%	32,6%
Sample 5	0,027	0,003	0,013	0,009	0,037	3	12,1%	48,1%
Sample 6	0,026					2		
Sample 7	0,202	0,010	0,016	0,028	0,046	3	5,0%	8,0%
Sample 8	0,226	0,010	0,020	0,027	0,055	3	4,3%	8,7%
Sample 9	0,055	0,004	0,015	0,010	0,042	3	6,7%	27,1%

GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,075					1		
Sample 2	0,021					1		
Sample 3	0,019					1		
Sample 4	0,067					1		
Sample 5								
Sample 6	0,025					1		
Sample 7	0,221					1		
Sample 8	0,238					1		
Sample 9	0,061					1		

NNK corrected								
LCMSMS SMNE	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,456	0,010	0,078	0,029	0,219	8	2,3%	17,2%
Sample 2	0,132	0,005	0,026	0,014	0,074	8	3,9%	20,0%
Sample 3	0,077	0,005	0,017	0,015	0,048	8	7,1%	22,0%
Sample 4	0,151	0,010	0,029	0,027	0,081	8	6,4%	19,3%
Sample 5	0,233	0,015	0,061	0,042	0,171	8	6,5%	26,2%
Sample 6	0,092	0,005	0,016	0,014	0,045	8	5,4%	17,3%
Sample 7	0,684	0,018	0,118	0,050	0,330	9	2,6%	17,3%
Sample 8	0,548	0,023	0,093	0,065	0,261	9	4,2%	17,0%
Sample 9	0,255	0,005	0,057	0,014	0,159	8	1,9%	22,3%

LCMSMS	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,484	0,038	0,040	0,105	0,112	3	7,8%	8,2%
Sample 2	0,128	0,008	0,007	0,022	0,021	3	6,2%	5,8%
Sample 3	0,086	0,017	0,023	0,048	0,063	3	20,1%	26,4%
Sample 4	0,148	0,015	0,014	0,041	0,038	3	9,8%	9,2%
Sample 5	0,234	0,023	0,079	0,065	0,221	3	10,0%	33,7%
Sample 6	0,096	0,015	0,017	0,042	0,047	3	15,7%	17,6%
Sample 7	0,688	0,026	0,024	0,074	0,067	3	3,8%	3,5%
Sample 8	0,577	0,016	0,035	0,044	0,099	3	2,8%	6,1%
Sample 9	0,282	0,016	0,025	0,044	0,069	3	5,5%	8,8%

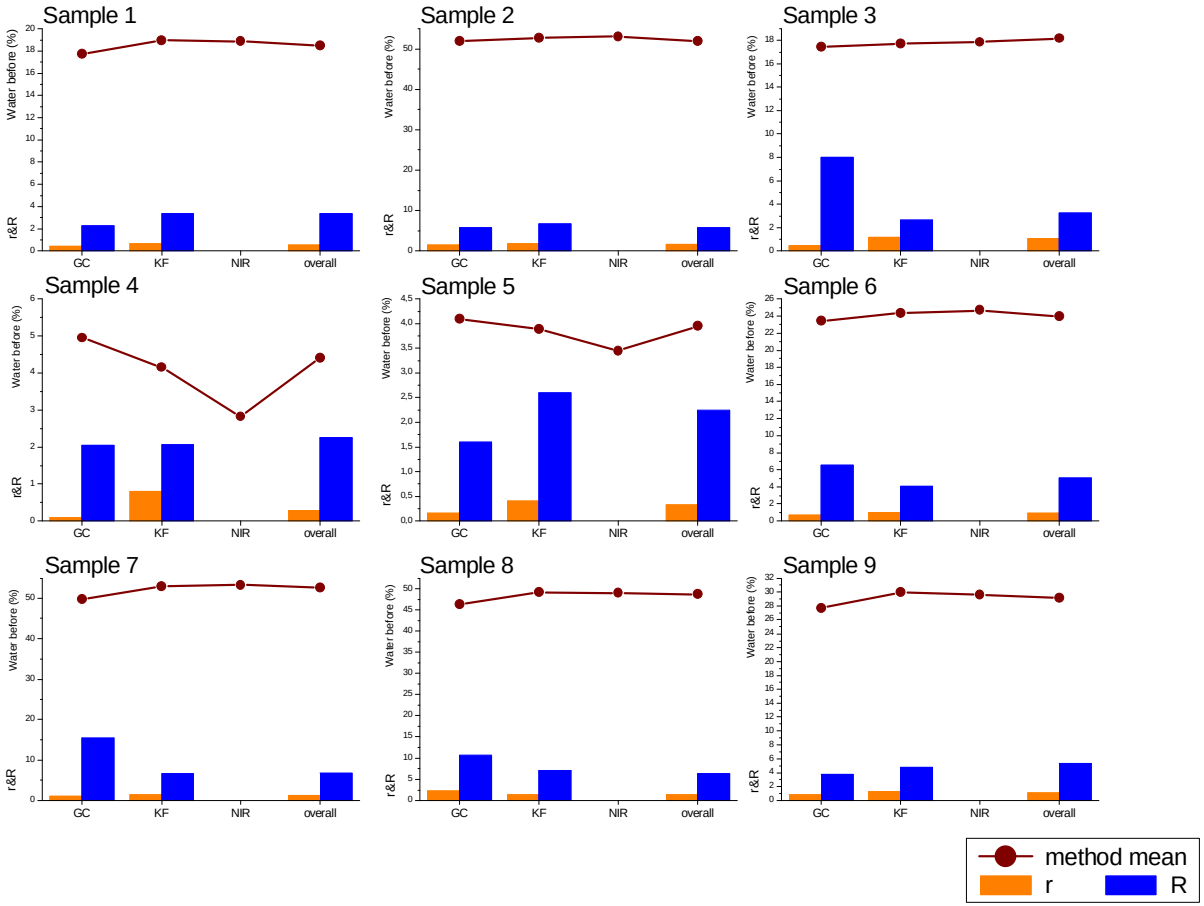
GC	Mean	r SD	R SD	r	R	N	CV r	CV R
Sample 1	0,744	0,054	0,255	0,152	0,715	3	7,3%	34,3%
Sample 2	0,300	0,014	0,227	0,038	0,635	3	4,6%	75,7%
Sample 3	0,090					2		
Sample 4	0,179					2		
Sample 5	0,252					2		
Sample 6	0,130					2		
Sample 7	0,930	0,015	0,198	0,043	0,556	3	1,7%	21,3%
Sample 8	0,776	0,021	0,203	0,058	0,568	3	2,7%	26,1%
Sample 9	0,372	0,011	0,085	0,030	0,239	3	2,8%	23,0%

Comparison of methods

This part is to visualise the method comparisons for Water (before and after), Nicotine, pH and TSNAs (as received and corrected).

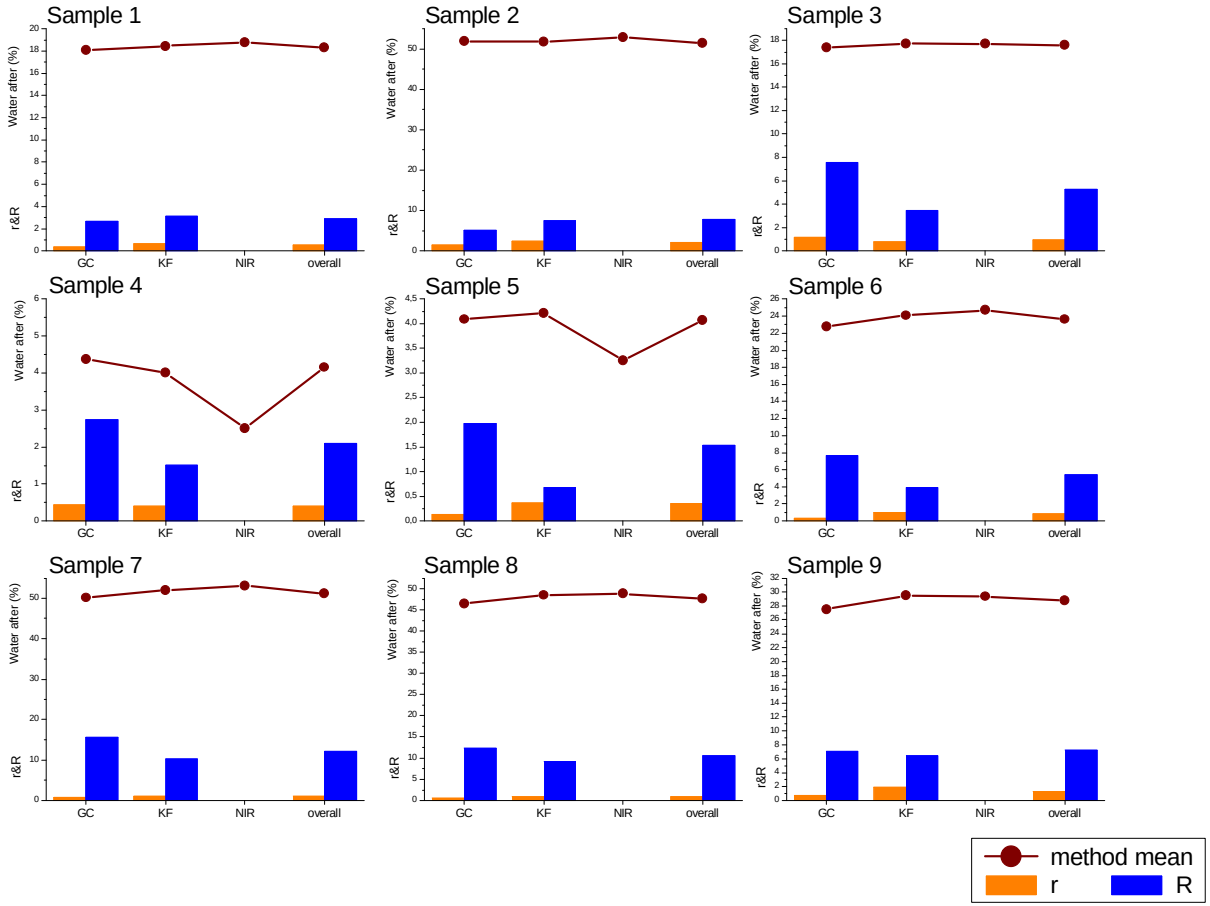
Each single graph shows the actual r&R figures for each method as bars and the method mean as dotted line. The overall r&R figures and the overall mean are also plotted

Comparison of r&R and means per method: Water before (%)



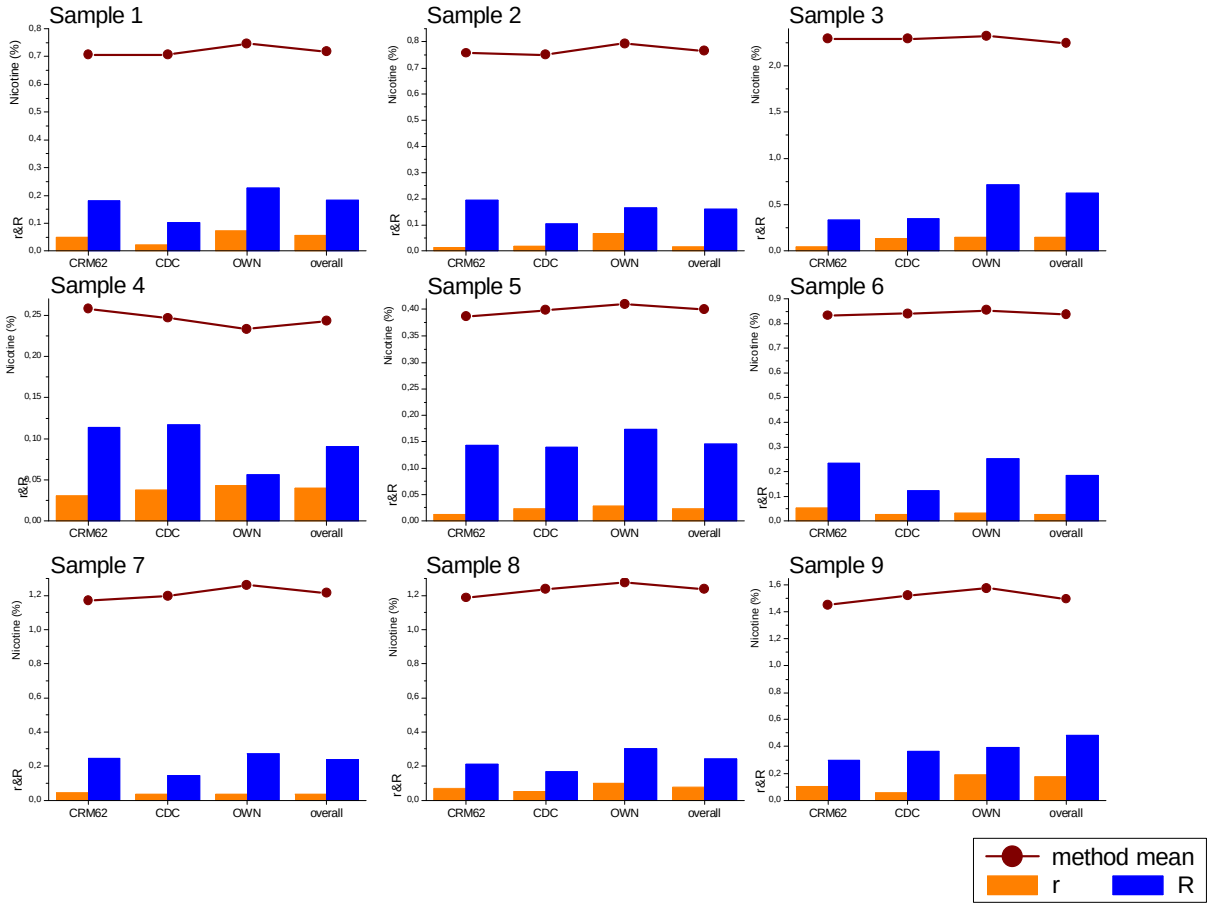
Graph Comp. 1: Comparison of r&R and means per method. Analyte: Water before (%).

Comparison of r&R and means per method: Water after (%)



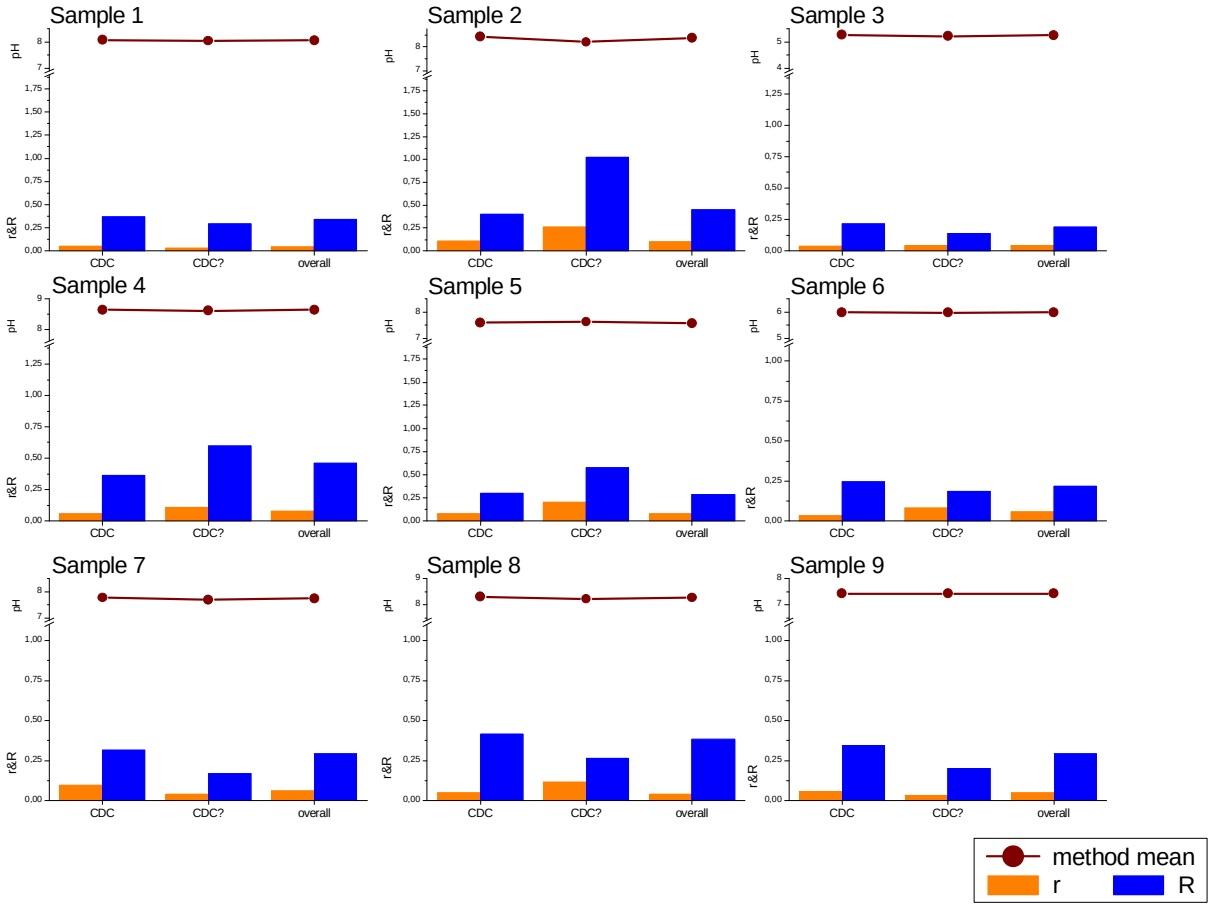
Graph Comp. 2: Comparison of r&R and means per method. Analyte: Water after (%).

Comparison of r&R and means per method: Nicotine(%)



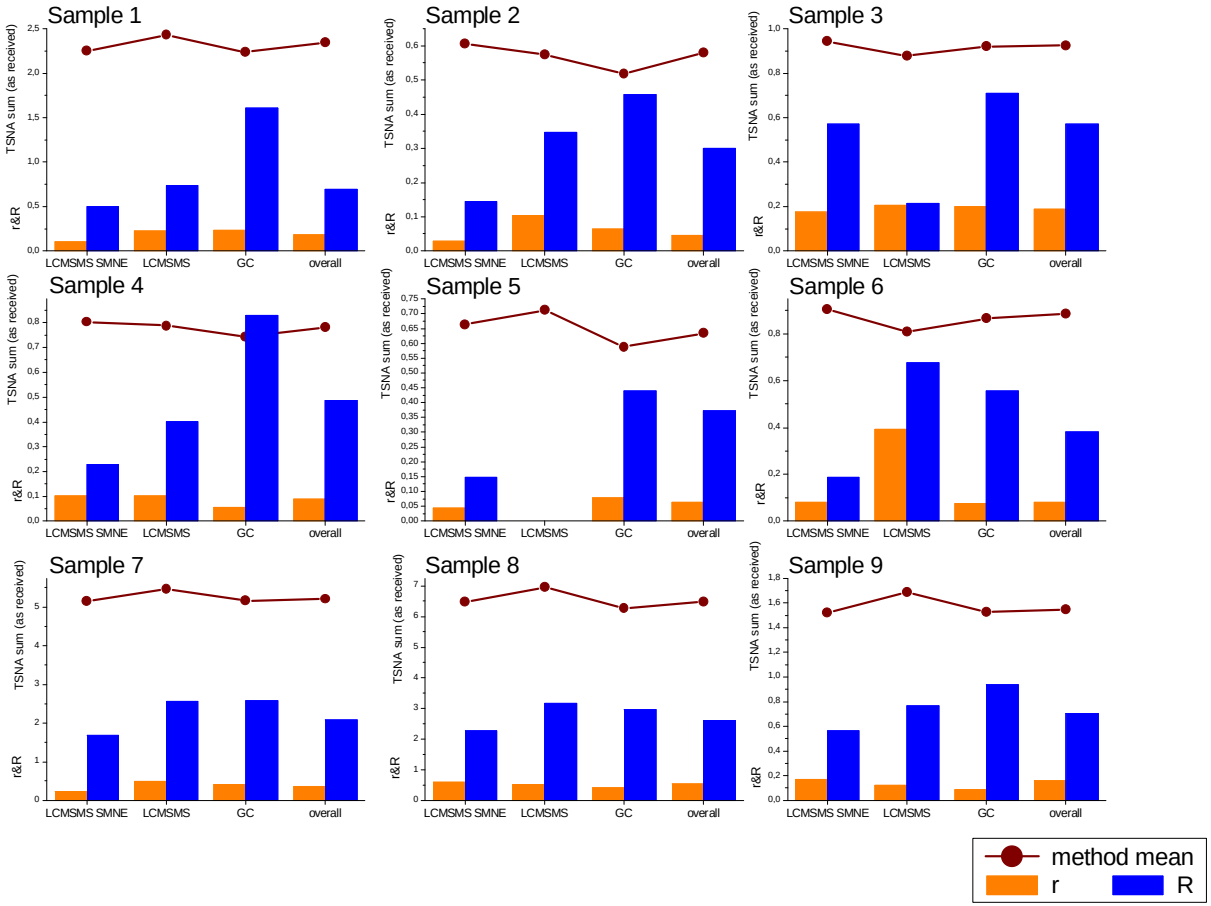
Graph Comp. 3: Comparison of r&R and means per method. Analyte: Nicotine (%).

Comparison of r&R and means per method: pH



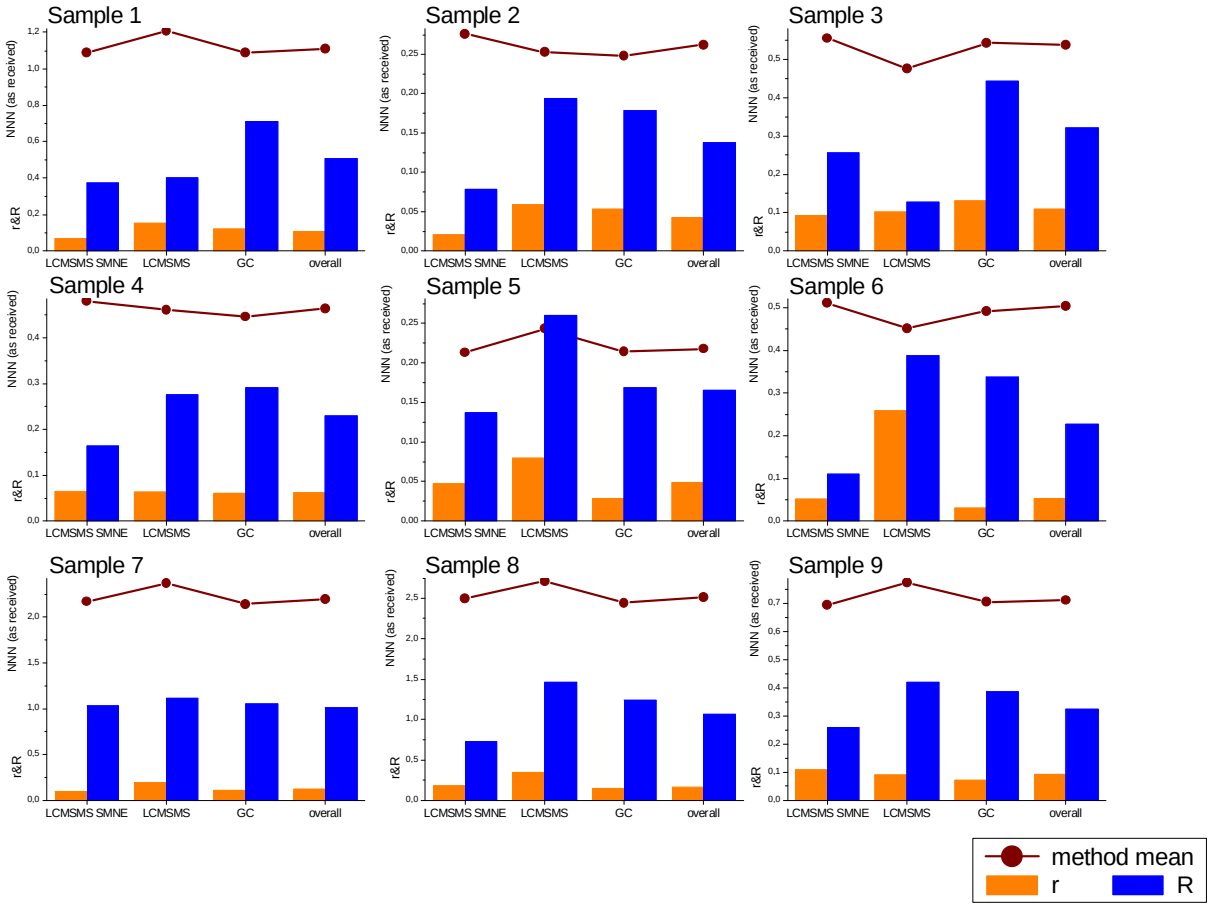
Graph Comp. 4: Comparison of r&R and means per method. Analyte: pH

Comparison of r&R and means per method: TSNA sum (as received)



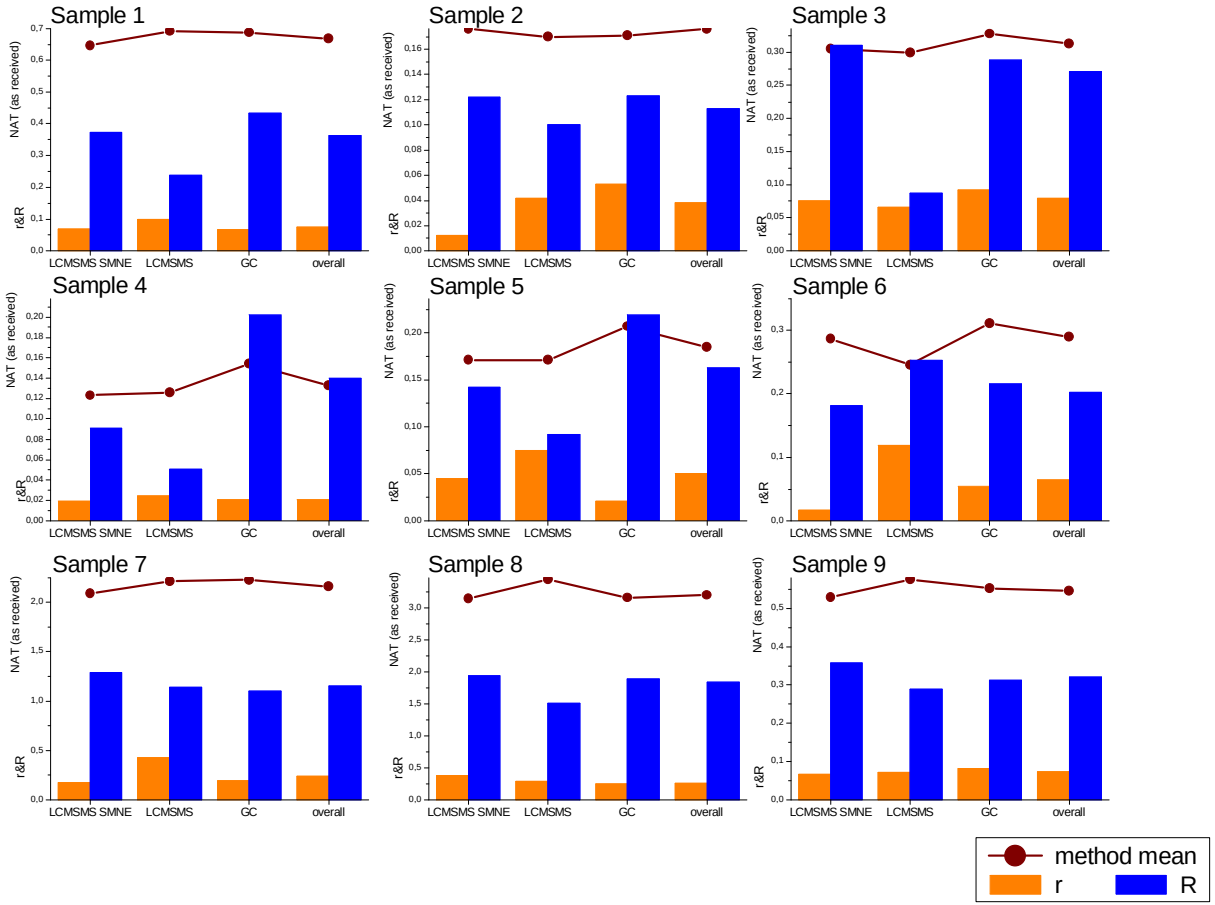
Graph Comp. 5: Comparison of r&R and means per method. Analyte: TSNA sum (as received).

Comparison of r&R and means per method: NNN (as received)



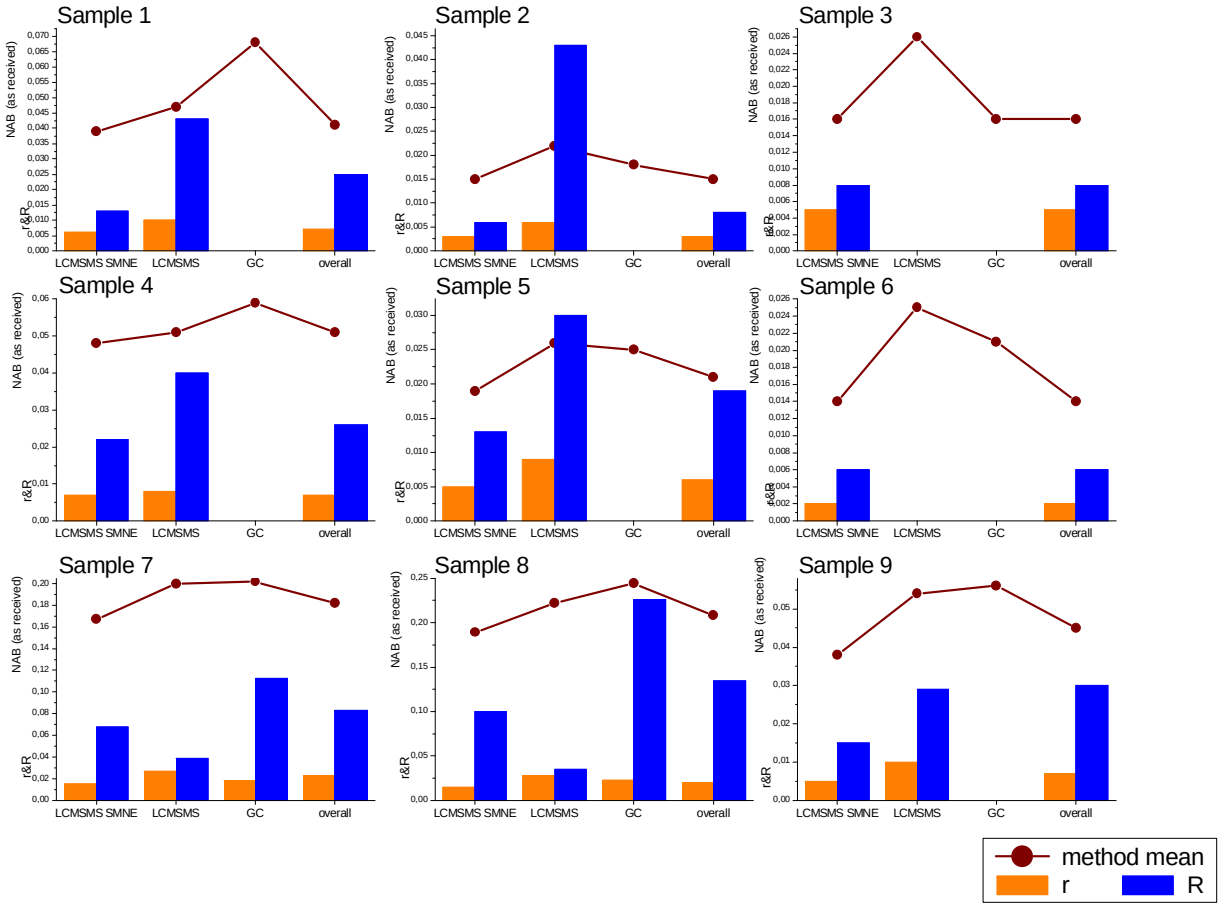
Graph Comp. 6: Comparison of r&R and means per method. Analyte: NNN (as received).

Comparison of r&R and means per method: NAT (as received)



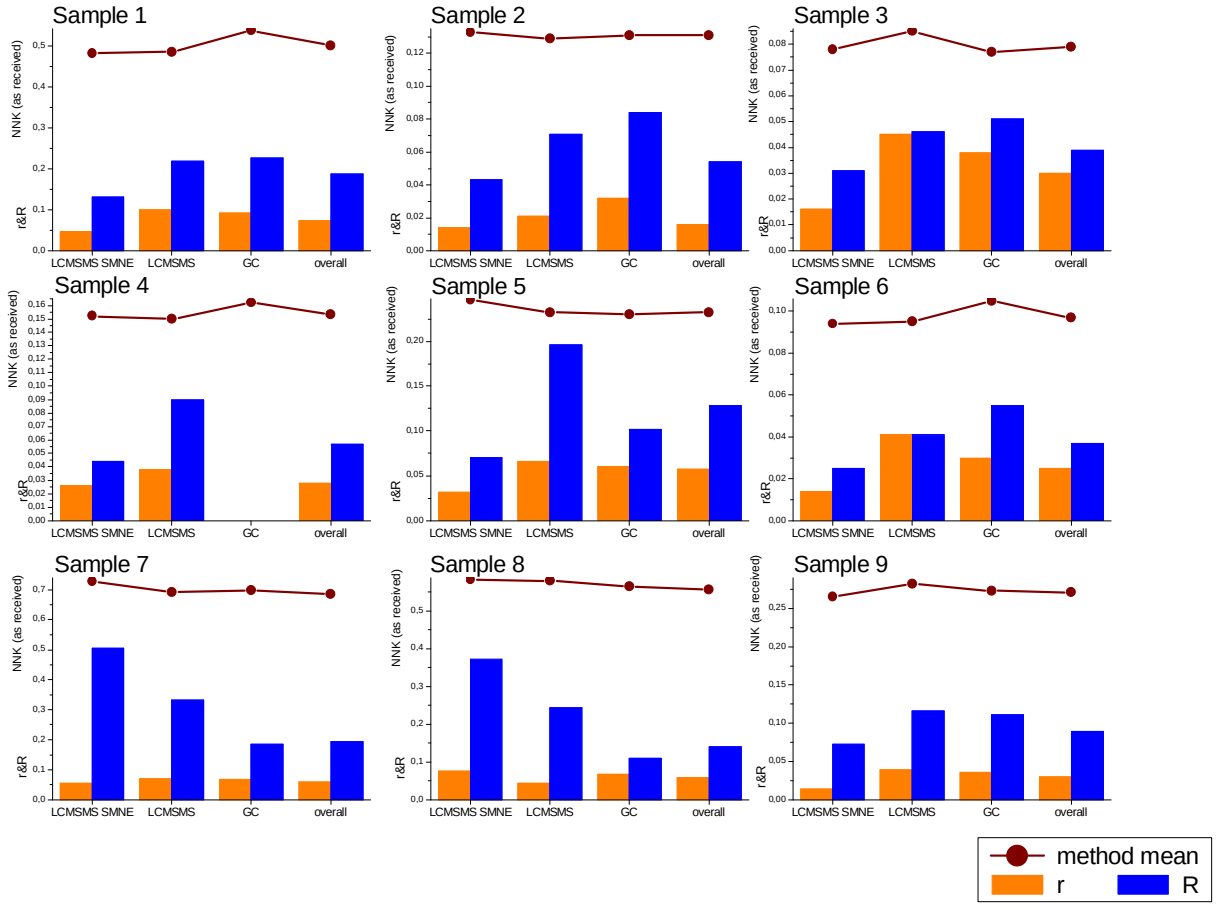
Graph Comp. 7: Comparison of r&R and means per method. Analyte: NAT (as received).

Comparison of r&R and means per method: NAB (as received)

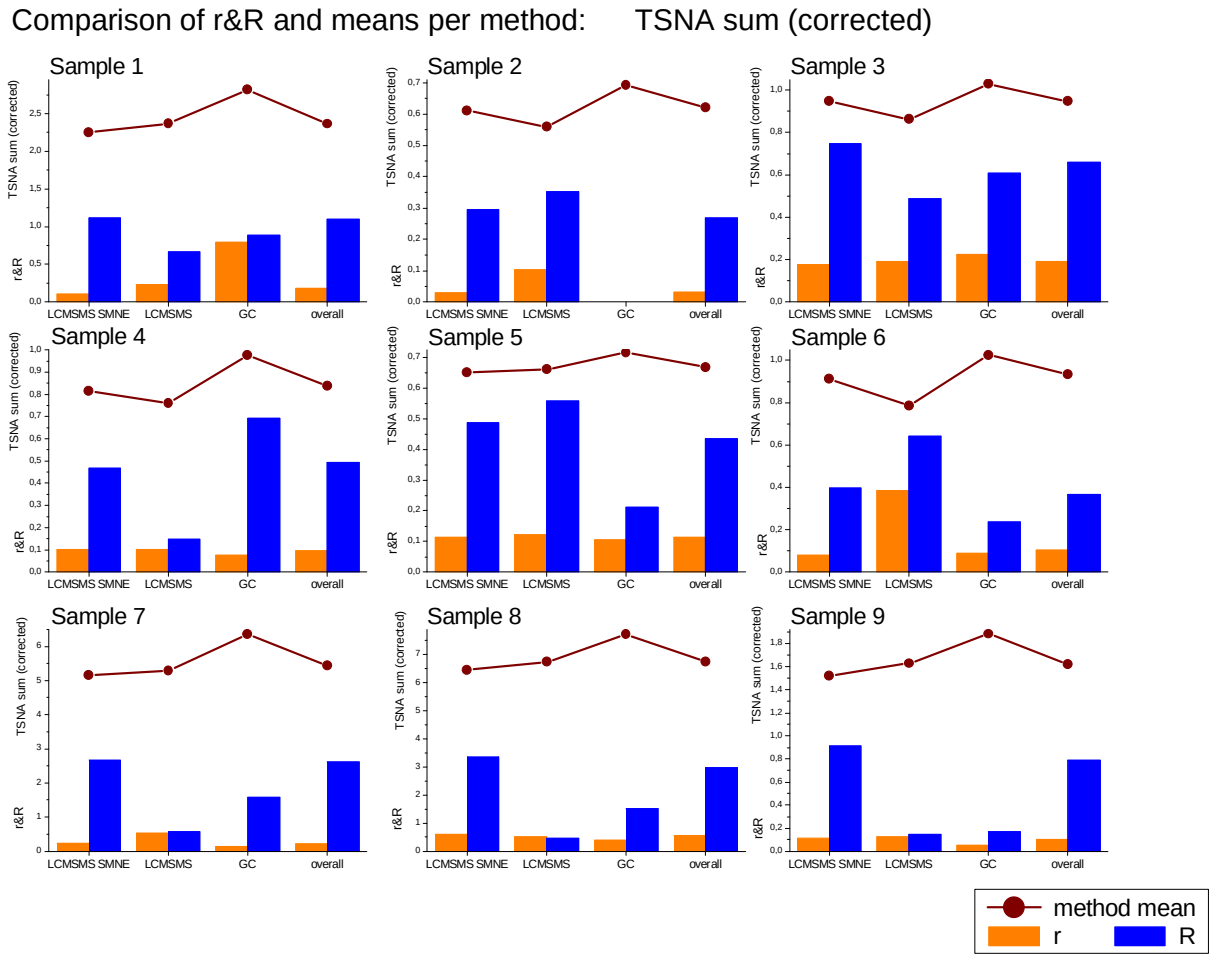


Graph Comp. 8: Comparison of r&R and means per method. Analyte: NAB (as received).

Comparison of r&R and means per method: NNK (as received)

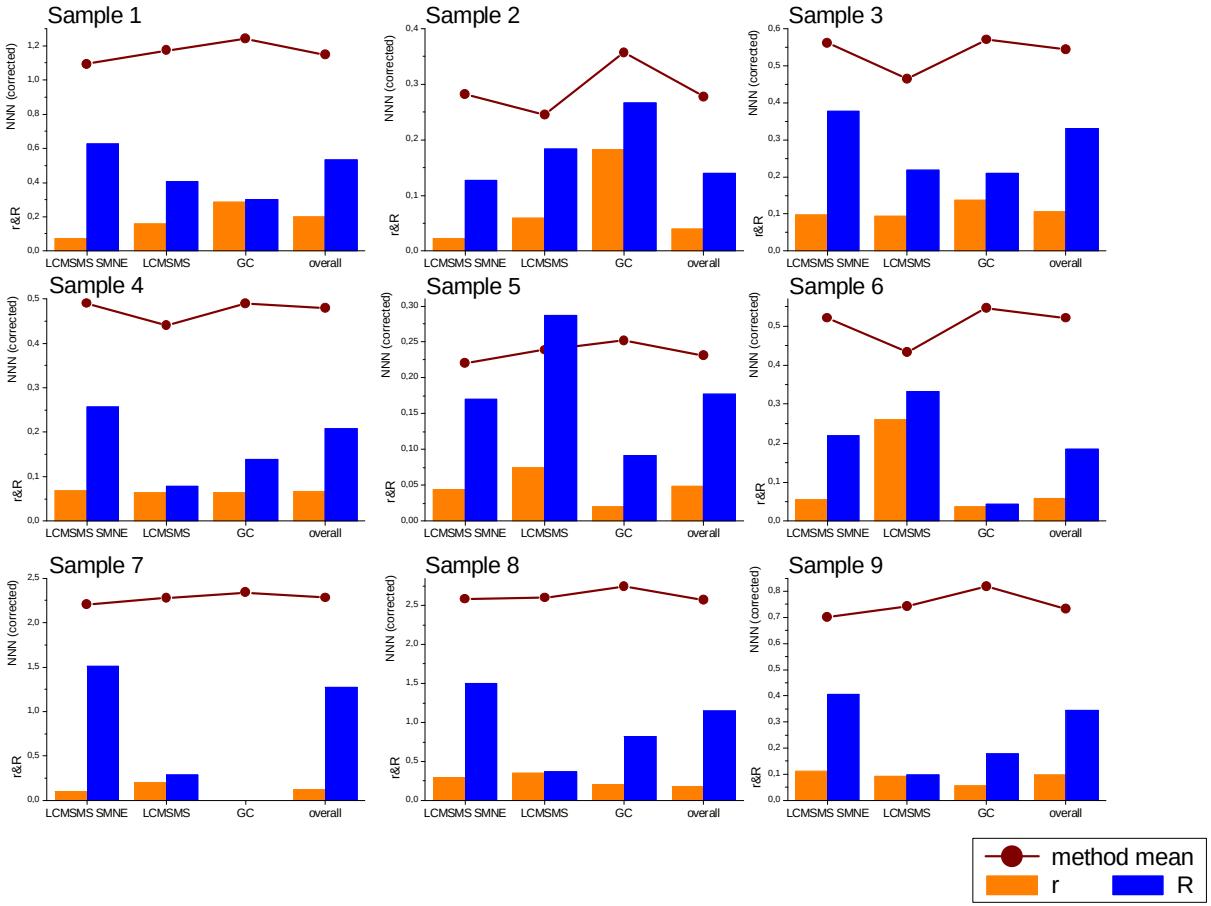


Graph Comp. 9: Comparison of r&R and means per method. Analyte: NNK (as received).



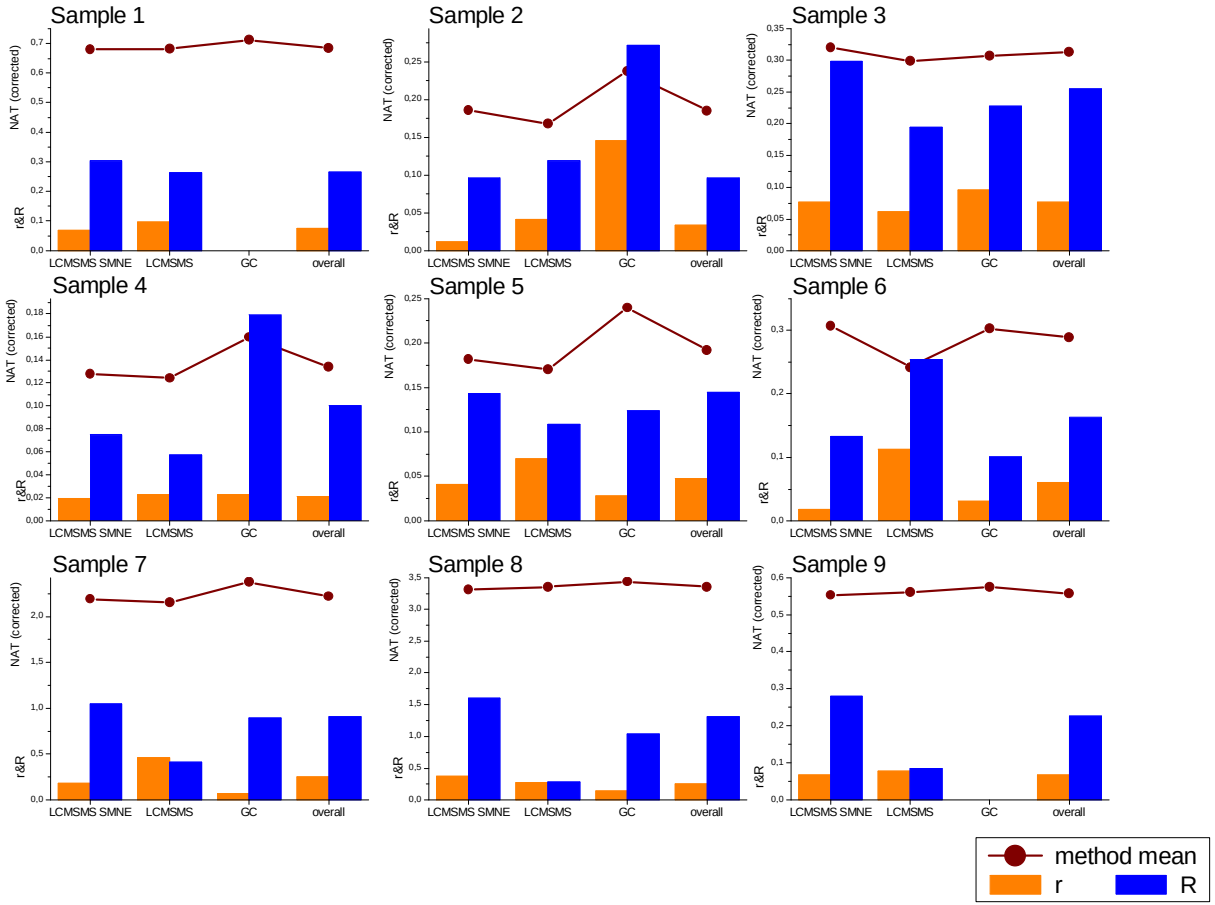
Graph Comp. 10: Comparison of r&R and means per method. Analyte: TSNA sum (corrected).

Comparison of r&R and means per method: NNN (corrected)



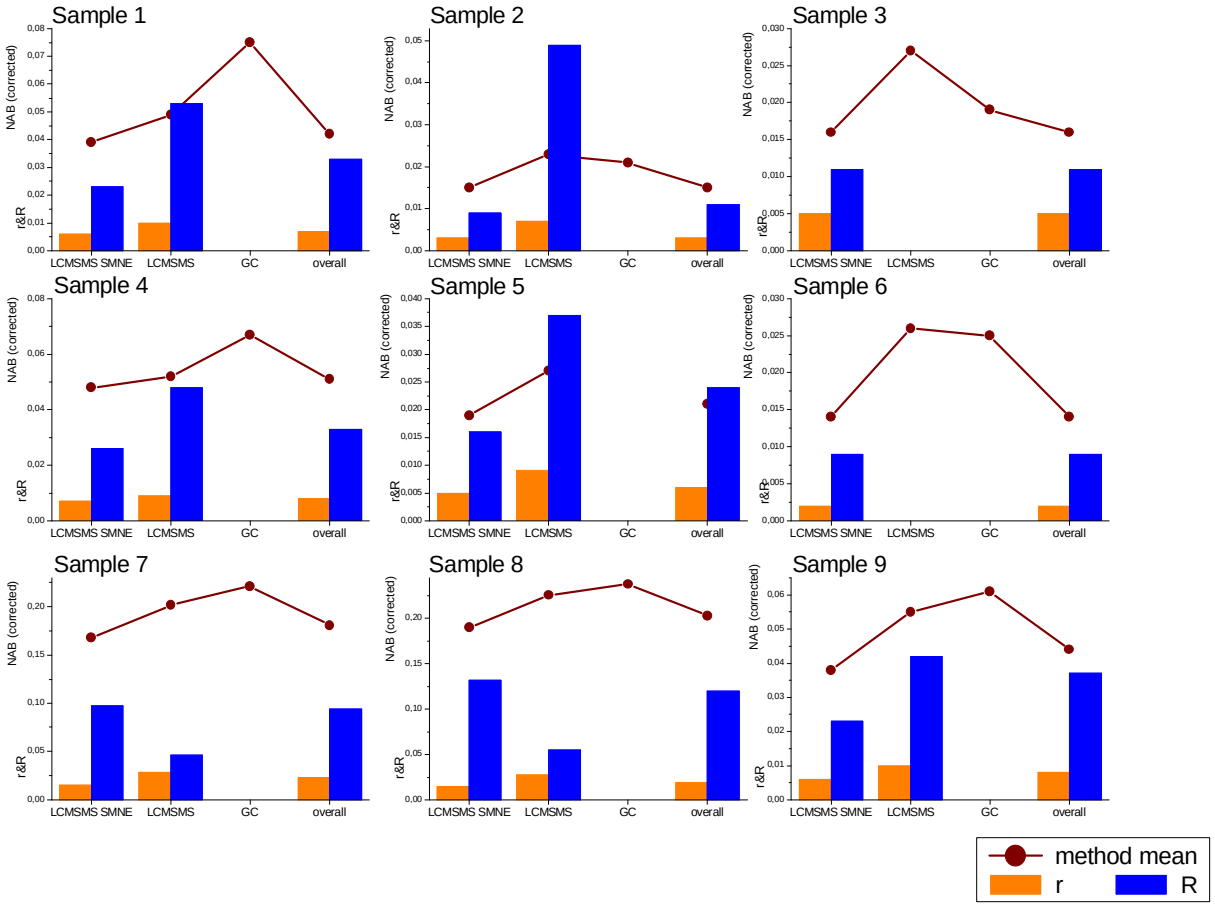
Graph Comp. 11: Comparison of r&R and means per method. Analyte: NNN (corrected).

Comparison of r&R and means per method: NAT (corrected)

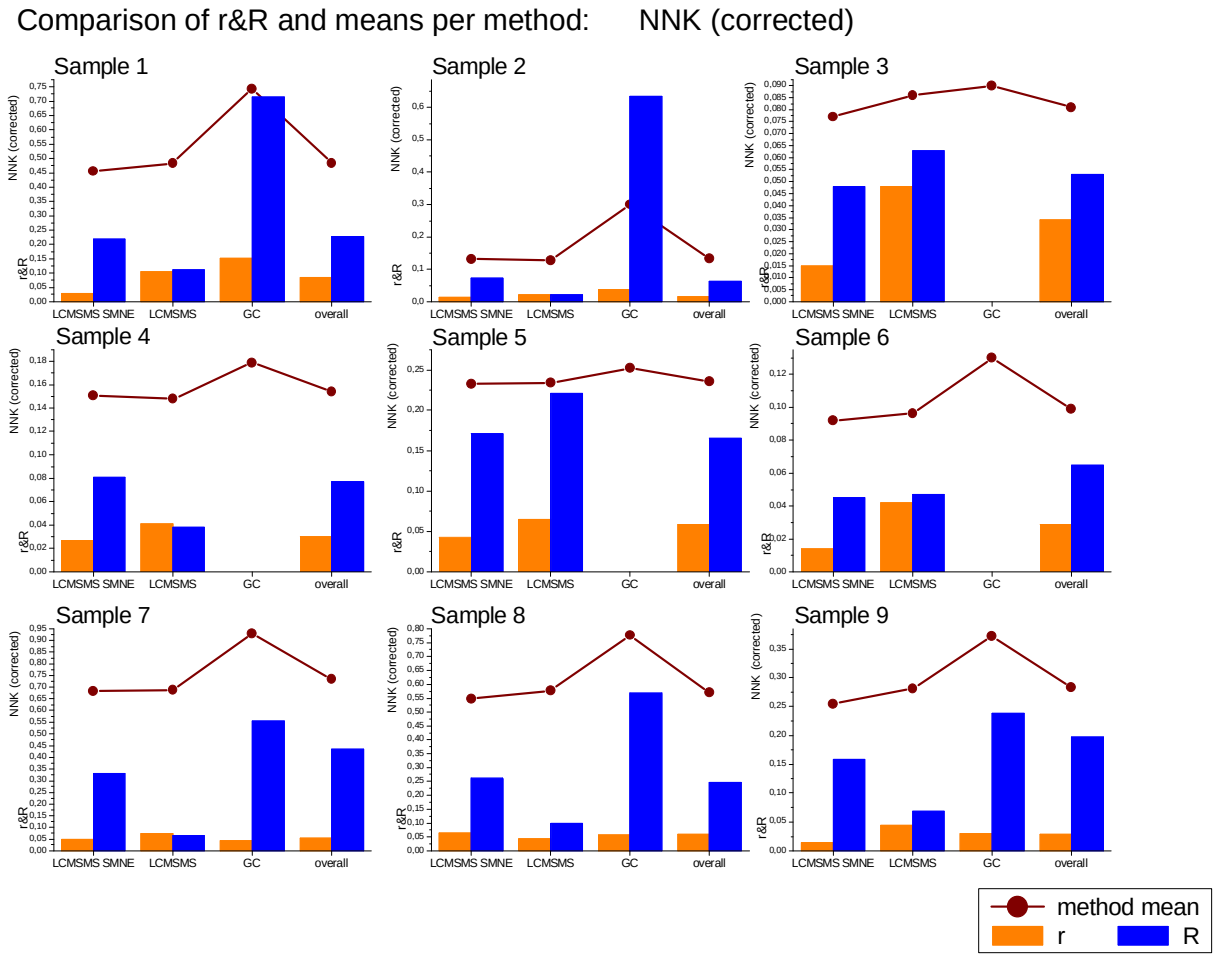


Graph Comp. 12: Comparison of r&R and means per method. Analyte: NAT (corrected).

Comparison of r&R and means per method: NAB (corrected)



Graph Comp. 13: Comparison of r&R and means per method. Analyte: NAB (corrected).



Graph Comp. 14: Comparison of r&R and means per method. Analyte: NNK (corrected).

z-scores and overall LAB performance

As mentioned in the preface of this report, a *z*-score indicates how many standard deviations an observation (a laboratories result) is above or below the overall mean. Outlying data was removed prior to the calculation of the *z*-scores.

The *z*-scores were used to derive an overall performance of a laboratory for a parameter (and method used). The overall LAB performance is calculated as the mean of *z*-scores divided by the square root of the number of *z*-scores of a laboratory for a certain parameter.

The following limits were set to evaluate the performance of a laboratory compared to the other.

Overall performance (OVP):

+4 < OVP	**UNSATISFACTORY HIGH**
+3 < OVP < +4	**QUESTIONABLE HIGH**
+2 < OVP < +3	**SATISFACTORY (HIGH)**
-2 < OVP < +2	**GOOD**
-3 < OVP < -2	**SATISFACTORY (LOW)**
-4 < OVP < -3	**QUESTIONABLE LOW**
OVP < -4	**UNSATISFACTORY LOW**

Water before												
	Lab Code	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**	**Sample7**	**Sample8**	**Sample9**	Performance	
GC CRM 57	**1**	0,058	-0,347	0,128	1,632	0,683	0,303	0,124	0,012	0,086	0,89	**GOOD**
KF OTHER	**2**	-0,009		-1,232		-1,391	-0,173	-0,222	0,139	0,337	-0,96	**GOOD**
GC OTHER	**3**	-1,742	-1,755		-1,635	-1,056	-2,901	-2,252	-1,233	-1,665	-5,03	**UNSATISFACTORY**
KF CRM 56	**4**											
	5											
	6											
GC CRM 57	**7**	-0,673	0,132	1,620	-0,157	0,136	1,038	-0,280	-0,261	-1,105	0,15	**GOOD**
GC CRM 57	**8**	-0,258	0,733	-0,053	1,243	0,795	-0,192	0,711		-0,328	0,94	**GOOD**
GC CRM 57	**9**	-0,194		0,200	1,142	0,829	-0,399			-1,552	0,01	**GOOD**
KF CRM 56	**10**	0,024	0,009	-0,908		-0,393	0,392	0,637	0,106	1,320	0,42	**GOOD**
KF OTHER	**11**	2,497	1,002	-0,088	1,093	2,106	0,673	0,861	1,383	1,507	3,68	**QUESTIONABLE**
GC CRM 57	**12**	-1,220	-0,201	2,299	-0,130	-0,137	0,040	-0,146	-0,642	-0,386	-0,17	**GOOD**
KF OTHER	**13**		0,428	-0,226	-0,422	-0,473	0,156	0,338	0,011	0,517	0,12	**GOOD**
KF OTHER	**14**	-0,217	-0,106	0,278	-1,135	-2,218	-0,937	-0,411	-0,469	0,784	-1,48	**GOOD**
	15											
KF CRM 56	**16**	0,376	0,374	0,121	0,136	0,336	0,471	0,649	0,491	0,283	1,08	**GOOD**
KF OTHER	**17**	0,276	0,116	0,140			0,470	0,252	0,409	-0,319	0,51	**GOOD**
KF OTHER	**18**	1,527	1,965	0,878	0,302	0,814	1,766	1,752	2,145	1,608	4,25	**UNSATISFACTORY**
KF CRM 56	**19**	0,560	0,229	0,133	0,077	0,459	0,457	0,397	0,651	0,165	1,04	**GOOD**
KF CRM 56	**20**	-1,291	-2,382	-1,689	-0,207	0,038	-1,311	-2,203	-2,228	-1,568	-4,28	**UNSATISFACTORY**
	21											
KF OTHER	**22**	-0,032	-0,451	-1,346	-0,235	0,099	-0,223	-0,450	-0,650	0,065	-1,07	**GOOD**
NIR transmittance	**23**	0,318	0,256	-0,254	-1,703	-0,628	0,372	0,241	0,135	0,252	-0,34	**GOOD**

Moisture

Lab Code	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Performance	
1	0,524	0,227	0,334	0,420	0,449	0,154	0,218	0,217	0,438	0,99	GOOD
2	0,124	-1,050	-1,319	-1,114	1,291	-1,360	-0,089	-0,540	0,317	-1,25	GOOD
3	0,554	-0,842	-1,682	-1,499	-1,531	-1,073	-1,334	-1,241	-1,678	-3,44	QUESTIONABLE
4	-1,093	0,119	-0,259	0,347	-0,391	-0,070	0,126	-0,330	-0,154	-0,57	GOOD
5	1,342	0,655	1,691	0,988	1,421	1,539	1,339	2,029	1,382	4,13	UNSATISFACTORY
6	0,193	0,582	0,090	0,286	-0,197	-0,078	0,843	-0,020	0,502	0,73	GOOD
7	-2,059	-1,306	-0,472	0,084	-0,146	-0,069	-0,023	0,207	-0,891	-1,56	GOOD
8	1,222	0,534	-0,079	0,645	-0,006	0,029	0,249	0,657	0,329	1,19	GOOD
9	0,036	0,777	0,507	0,593	1,000	0,450	0,416	0,010	-0,735	1,02	GOOD
10		2,264	2,020	1,788	1,652	2,005	1,569	1,703	1,505	5,13	UNSATISFACTORY
11											
12	-0,257	0,263	-0,286	-0,070	-0,498	-0,233	-0,641	0,341	0,074	-0,44	GOOD
13	0,367	0,072	-0,178	0,104	-0,368	-0,193	-0,239	-0,158	-0,355	-0,32	GOOD
14	0,767	0,918	0,696	1,117	0,432	0,492	1,275	0,395	0,376	2,16	SATISFACTORY
15	-0,189	-0,028	-0,056	-0,634	-1,437	-0,065	-1,954	-1,128	-0,893	-2,13	SATISFACTORY
16											
17	-0,988	-1,113	-0,413	-1,016	-0,588	-1,140	-1,222	-1,302	-1,526	-3,10	QUESTIONABLE
18											
19	-1,405	-1,867	-1,816	-2,313	-0,879	-1,700	-0,690	-1,908	-0,198	-4,26	UNSATISFACTORY
20	-0,405	-0,154	-0,488	-0,279	-1,832	-0,583	-0,562	-0,624		-1,74	GOOD
21	-0,954	-1,205	-0,224	-0,957	-0,058	-0,727	-1,266	-0,314	-1,155	-2,29	SATISFACTORY
22	1,924	1,227	1,511	0,802	1,227	1,828	0,691	0,764	1,916	3,96	QUESTIONABLE
23	0,296	-0,075	0,423	0,708	0,459	0,795	1,293	1,243	0,747	1,96	GOOD

pH

	Lab Code	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Performance	
CDC	**1**	0,476	0,404	0,549	0,562	-0,826	-0,785	-0,163	-0,079	-0,092	0,02	**GOOD**
CDC?	**2**	-0,581	-2,124	-0,070	0,697	-1,107	-0,793	-1,284	-1,272	-0,406	-2,31	**SATISFACTORY**
CDC	**3**	1,047	-0,337		-0,393	0,340	2,026	-0,299			0,97	**GOOD**
CDC	**4**	0,446	1,146	1,345	-0,007	0,609	1,291	1,127	0,956		2,44	**SATISFACTORY**
CDC	**5**	-1,329	0,734	0,411	-0,068	-0,348	0,513	-1,044	-1,105	-0,335	-0,86	**GOOD**
OTHER	**6**	-0,958	0,808	-0,252	0,581	1,679	1,096	-0,121	-0,380	0,683	1,05	**GOOD**
CDC	**7**	0,337	-0,090	-0,227	-1,267	0,579	-0,395	-0,202	0,186	0,832	-0,08	**GOOD**
CDC	**8**	1,637	0,909	2,720	1,453	1,386	0,526	2,390	2,789	1,721	5,18	**UNSATISFACTORY**
CDC	**9**	1,265	1,125	0,706	0,318	1,536	1,205	1,580	0,956	0,832	3,17	**QUESTIONABLE**
CDC?	**10**	0,774	1,043	-0,423	0,176	-0,766	-1,001	-0,007	-0,037	0,119	-0,04	**GOOD**
	11											
CDC	**12**	-0,100	-1,016	-0,080		-1,364	-1,563	1,288	-0,286	0,249	-1,02	**GOOD**
CDC?	**13**	-0,264	-0,460	-0,276	-1,623	-0,408	0,167	-0,121	-0,348	-0,157	-1,16	**GOOD**
CDC	**14**	-1,958	-1,201	-1,602	-0,840	-1,305	-1,131	-0,526	-0,609	-2,572	-3,91	**QUESTIONABLE**
CDC?	**15**	-0,810	-0,872	-0,866	1,172	-1,125	-0,828	-1,174	-0,782	-0,367	-1,88	**GOOD**
CDC?	**16**	-0,603	-0,820	-0,040	-1,524		-0,054	-0,957	-0,502	-0,384	-1,73	**GOOD**
	17											
CDC?	**18**			-1,553	-1,491		-1,347				-2,54	**SATISFACTORY**
CDC	**19**			-0,031	0,278	-0,288	0,167			-0,465	-0,15	**GOOD**
CDC?	**20**			0,706	0,054	0,938	1,118	-0,623		0,119	0,94	**GOOD**
CDC	**21**	0,118	1,249	0,215	1,050	0,071	-0,136	-0,040	-0,484	-1,113	0,31	**GOOD**
CDC?	**22**	1,285	-0,073		1,638		-0,024	0,061	0,832	1,536	1,99	**GOOD**
CDC	**23**	-0,305	-0,020	-0,684	-0,204	-0,426	-0,837	-0,049	0,084	-0,293	-0,91	**GOOD**

Nicotine												
	Lab Code	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**	**Sample7**	**Sample8**	**Sample9**	Performance	
CRM62 HEX	**1**	-0,384	-0,574	-0,294	-0,477	-0,100	-0,329	-0,494	-0,272	-0,828	-1,25	**GOOD**
?	**2**	-0,589	-0,589	-1,048	-0,013		-0,323	-0,469	-0,246	-0,041	-1,17	**GOOD**
OWN	**3**	-0,150	-0,248	-0,349	-0,890	0,203	-0,628	-0,013	0,088	-0,424	-0,80	**GOOD**
CDC	**4**				-1,156	-1,490				-2,244	-2,82	**SATISFACTORY**
?	**5**	-0,446	-0,654	-1,145	-0,519	-0,364	-0,250	-0,355	-0,087	-0,133	-1,32	**GOOD**
CDC	**6**	0,431	0,406	0,506	1,792	0,982	0,488	0,256	0,370	-2,011	1,07	**GOOD**
CDC?	**7**	-0,215	-0,455	-0,015	-0,781	-0,198	-0,332	-0,315	0,045	0,044	-0,74	**GOOD**
CDC	**8**	-0,056	-0,357	0,350	-0,894	-0,175	-0,045	0,056	0,266	0,221	-0,21	**GOOD**
CRM62 MTBE	**9**	0,309	0,396	0,486	0,670	0,521	0,405	-0,126	-0,005	-0,016	0,88	**GOOD**
OWN	**10**	0,137	0,376	0,349	-0,460	0,061	-0,519	-0,229	-0,015	0,317	0,01	**GOOD**
OWN	**11**	-0,012	-0,528	0,222	-0,536	-0,188	-0,215	-0,221	-0,143	-0,017	-0,55	**GOOD**
CDC	**12**	-0,448	-0,800	-0,578	0,501	0,017	-0,537	-0,688	-0,381	-0,758	-1,22	**GOOD**
CDC	**13**	-0,206	-0,528	0,225	-0,144	-0,388		-0,312	-0,017	-0,026	-0,49	**GOOD**
CRM62	**14**	0,326	0,200	0,233	1,319	-1,215	0,458	0,264	0,176	0,079	0,61	**GOOD**
OWN TNA?	**15**	0,861	1,005	-0,204	2,162	1,816	0,867	0,573	0,495	0,224	2,60	**SATISFACTORY**
CRM62 MTBE	**16**	2,681	**3,603**	2,620	2,230	2,574	2,806	**3,192**	2,169	2,512	8,13	**UNSATISFACTORY**
OWN	**17**	0,126	-0,214	0,055	-0,241	0,619	-0,119	-0,031	0,123	0,196	0,17	**GOOD**
OWN	**18**	1,398	1,491	2,047	-0,190	1,059	1,514	1,676	1,105	1,706	3,93	**QUESTIONABLE**
OWN	**19**	0,495		0,278	0,155	-0,051		-0,031	0,003	0,246	0,41	**GOOD**
CRM62 HEX MOD	**20**	-0,731	-1,355	-1,056	-0,830	-0,707	-1,065	-1,088	-0,583	-0,805	-2,74	**SATISFACTORY**
CDC?	**21**	-0,248	-0,685	0,318	-0,098	-0,178	0,224	-0,084	0,062	0,985	0,10	**GOOD**
OWN	**22**	-0,899	0,016	0,123	-0,637	-0,041	0,352	0,641	-0,167	0,283	-0,11	**GOOD**
OWN	**23**	0,569		-0,898	-0,960	-2,035		0,152	0,790	0,490	-0,72	**GOOD**

TSNA sum (as recieved)												
	Lab Code	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**	**Sample7**	**Sample8**	**Sample9**	Performance	
LCMSMS SMNE	**1**	-0,008	0,357	0,140	0,334	0,131	0,401	0,354	0,233	0,509	0,82	**GOOD**
LCMSMS SMNE	**2**	-1,143	-0,398	-0,026	-0,677	-0,297	-0,197	-0,307	-0,184	-0,535	-1,25	**GOOD**
LCMSMS SMNE	**3**		0,663	0,644	0,089		0,306	0,165	-0,022	-0,057	0,68	**GOOD**
LCMSMS SMNE	**4**	-0,547		-2,266				-0,838	-0,607	-1,501	-2,58	**SATISFACTORY**
GC TEA	**5**		**-3,216**	-2,142	-2,314	-2,782	**-3,175**	-2,768	-2,536	-2,599	-7,61	**UNSATISFACTORY**
GC NCD	**6**	-0,800	-0,179	-1,092	-1,010	-0,676	-0,415	0,644	0,224	-0,239	-1,18	**GOOD**
LCMSMS SMNE	**7**	0,289	0,742	0,675	0,451	0,546	0,570	0,733	0,947	0,955	1,97	**GOOD**
LCMSMS	**8**	0,957		-0,392	-0,400	1,815		0,256	0,244	0,508	1,13	**GOOD**
LCMSMS	**9**	-0,834	0,251	-0,017	-0,465	-0,640	-0,192	-0,821	-0,516	-0,481	-1,24	**GOOD**
LCMSMS	**10**	0,996	0,871	-0,314	0,980			1,632	1,865	1,717	2,93	**SATISFACTORY**
	11											
GC TEA	**12**		0,506	-0,266	0,495	0,684	-0,155	0,169	0,037	0,854	0,82	**GOOD**
LCMSMS SMNE	**13**	0,694	0,605	1,229	0,295	0,613	0,490	0,939	1,003	0,581	2,15	**SATISFACTORY**
LCMSMS SMNE	**14**	-1,259	-0,230	-0,242	0,239	-0,167	-0,612	-1,364	-1,596	-0,808	-2,01	**SATISFACTORY**
GC TEA	**15**	1,012		0,625		0,356	0,030	-0,153	-0,189	-0,539	0,43	**GOOD**
GC TEA	**16**	-0,933	-0,552	0,159		-0,563	0,315	0,344	0,501	-0,146	-0,31	**GOOD**
LCMSMS SMNE	**17**	-0,080	0,458	0,771	0,645	0,479	0,577	0,541	0,824	0,459	1,56	**GOOD**
GC TEA	**18**	0,567	0,465	1,139	-0,583	0,364	1,161	0,381	-0,664	0,634	1,15	**GOOD**
	19											
	20											
GC TEA	**21**	2,154		1,410	2,287	0,135	1,246	0,963	1,025	1,104	3,65	**QUESTIONABLE**
	22											
LCMSMS SMNE	**23**	-1,066	-0,345	-0,037	-0,366		-0,351	-0,871	-0,588	-0,417	-1,43	**GOOD**

TSNA sum (corrected)												
	Lab Code	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**	**Sample7**	**Sample8**	**Sample9**	Performance	
LCMSMS SMNE	**1**	-0,495	-0,492	-0,286	-0,347	-0,416	-0,508	-0,381	-0,525	-0,264	-1,24	**GOOD**
LCMSMS SMNE	**2**	-0,805	-0,904	-0,157	-1,025	-0,506	-0,655	-0,519	-0,477	-0,787	-1,95	**GOOD**
LCMSMS SMNE	**3**		0,179	0,357	-0,331	0,412	-0,216	-0,218	-0,410		-0,09	**GOOD**
LCMSMS SMNE	**4**	-1,042		-2,283				-1,459	-1,445	-2,065	-3,71	**QUESTIONABLE**
GC TEA	**5**											
GC NCD	**6**	0,696		-0,332	-0,533	0,009	0,648	1,650	1,369	0,699	1,49	**GOOD**
LCMSMS SMNE	**7**	0,187	0,471	0,521	0,166	0,303	0,282	0,405	0,634	0,617	1,20	**GOOD**
LCMSMS	**8**	0,545		-0,434	-0,717	1,361		-0,027	-0,047	0,169	0,32	**GOOD**
LCMSMS	**9**	0,118	0,652	0,389	-0,280	-0,317	0,254		-0,002	-0,066	0,26	**GOOD**
LCMSMS	**10**	-0,610	-0,697	-1,041	-0,326	-1,181	-0,983	-0,166	-0,014	-0,013	-1,68	**GOOD**
	11											
GC TEA	**12**		0,785	0,043	0,645	0,840	0,107	0,456	0,370	1,070	1,53	**GOOD**
LCMSMS SMNE	**13**	1,091	0,997	1,515	0,494	0,837	0,954	1,209	1,396	0,891	3,13	**QUESTIONABLE**
LCMSMS SMNE	**14**	0,583	0,898	0,732	1,187	0,731	0,679	-0,085	-0,372	0,305	1,55	**GOOD**
GC TEA	**15**											
GC TEA	**16**											
LCMSMS SMNE	**17**	0,174	0,393	0,776	0,547	0,412	0,566	0,472	0,776	0,394	1,50	**GOOD**
GC TEA	**18**											
	19											
	20											
GC TEA	**21**	1,672		1,384	2,286	0,129	1,359	0,864	1,010	1,027	3,44	**QUESTIONABLE**
	22											
LCMSMS SMNE	**23**	-2,112	-2,282	-1,184	-1,765	-2,614	-2,487	-2,202	-2,262	-1,976	-6,29	**UNSATISFACTORY**

NNN (as received)

	Lab Code	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Performance	
LCMSMS SMNE	1	0,378	0,654	0,168	0,565	0,084	0,521	0,381	0,371	0,425	1,18	GOOD
LCMSMS SMNE	2	-0,523	-0,276	-0,235	-0,984	-0,444	-0,365	-0,188	-0,208	-0,560	-1,26	GOOD
LCMSMS SMNE	3		0,244	0,847	0,059	0,332	0,127	-0,011	0,182	-0,492	0,46	GOOD
LCMSMS SMNE	4	-1,213		-1,349				-2,211	-1,091	-1,494	-3,29	QUESTIONABLE
GC TEA	5	-2,468	-2,795	-2,186	-2,808	-1,731	-3,055	-2,219	-2,539	-2,282	-7,36	UNSATISFACTORY
GC NCD	6	-0,983	-0,476	-1,218	-1,099	-0,930	-1,023	0,121	-0,607	-0,673	-2,30	SATISFACTORY
LCMSMS SMNE	7	0,228	0,685	0,426	0,661	0,438	0,160	0,448	0,748	0,661	1,49	GOOD
LCMSMS	8	0,983	-1,794	-0,902	-0,473	2,227		0,328		0,119	0,18	GOOD
LCMSMS	9	-0,323	0,343	-0,411	-0,971	-0,720	-0,512	-0,529	-0,420	-0,462	-1,34	GOOD
LCMSMS	10	0,989	0,870	-0,378	1,345	-0,209	0,681	1,646	2,068	2,039	3,02	QUESTIONABLE
	11											
GC TEA	12		0,559	-0,344	0,324	0,398	-0,143	0,290	0,205	0,802	0,74	GOOD
LCMSMS SMNE	13	0,916	0,546	1,340	0,332	0,261	0,407	1,453		0,568	2,06	SATISFACTORY
LCMSMS SMNE	14	0,526	1,187	0,621	1,183	0,792	0,666	0,504	0,549	0,613	2,21	SATISFACTORY
GC TEA	15	0,536	0,163	1,014	0,546	0,073	0,429	-0,037	-0,225	-0,418	0,69	GOOD
GC TEA	16	-0,076	0,012	0,160	0,441	-0,361	0,341	0,392	0,429	0,113	0,48	GOOD
LCMSMS SMNE	17	-0,506	-0,210	-0,044	0,396	-0,301	-0,085	-0,080	0,019	-0,252	-0,35	GOOD
GC TEA	18	1,062	0,737	1,361	0,702	0,420	1,054		1,024	1,023	2,61	SATISFACTORY
	19											
	20											
GC TEA	21	1,248		1,555	0,349	1,537	1,419	0,578	0,460	1,103	2,92	SATISFACTORY
	22											
LCMSMS SMNE	23	-0,776	-0,448	-0,425	-0,567	-1,869	-0,621	-0,865	-0,965	-0,834	-2,46	SATISFACTORY

NAT (as received)

	Lab Code	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Performance	
LCMSMS SMNE	1	-0,274	0,123	0,001	-0,174	-0,406	-0,014	0,281	0,121	0,369	0,01	GOOD
LCMSMS SMNE	2	-0,423	-0,072	0,183	-0,447	-0,186	0,031	-0,120	-0,006	-0,422	-0,49	GOOD
LCMSMS SMNE	3	0,170	1,125	0,001	-0,392	0,254	0,253	0,080		0,270	0,62	GOOD
LCMSMS SMNE	4	0,384		-2,355				-0,660	-0,649	-1,221	-2,01	SATISFACTORY
GC TEA	5	-1,486	-1,693	-1,115	-1,069	-1,089	-1,249	-1,522	-1,455	-1,392	-4,02	UNSATISFACTORY
GC NCD	6	0,380	0,026	-0,777	-0,694	0,204	-0,068	1,416	0,942	0,587	0,67	GOOD
LCMSMS SMNE	7	0,561	0,811	0,682	0,273	0,425	0,570	0,916	0,849	1,068	2,05	SATISFACTORY
LCMSMS	8	0,782	-1,120	0,026	-0,495	0,217	-1,947	0,149	0,319	0,434	-0,55	GOOD
LCMSMS	9	-0,449	0,119	-0,032	-0,212	-0,513	-0,286	-0,817	-0,419	-0,688	-1,10	GOOD
LCMSMS	10	0,222	0,535	-0,421	0,188	-0,453	0,339	1,076	1,211	1,090	1,26	GOOD
	11											
GC TEA	12		0,085	-0,523	0,052	0,685	-0,522	-0,058	-0,112		-0,15	GOOD
LCMSMS SMNE	13	0,097	0,441	0,714	-0,157	0,345	0,129	0,334	0,575	0,303	0,93	GOOD
LCMSMS SMNE	14	-2,650	-2,310	-1,578	-1,517	-1,702	-2,021	-2,752	-2,483	-2,509	-6,51	UNSATISFACTORY
GC TEA	15	0,742	1,233	0,898	0,430	0,479	0,922	-0,096	0,107	-0,473	1,41	GOOD
GC TEA	16	-0,452	-0,367	0,169		-0,655	0,243	0,471	0,544	0,013	-0,01	GOOD
LCMSMS SMNE	17	0,941	1,194	1,321	0,802	0,636	0,875	1,003	1,078	0,983	2,94	SATISFACTORY
GC TEA	18	-0,298		0,633	0,589	0,260	0,696	-0,137	-1,444	0,228	0,19	GOOD
	19											
	20											
GC TEA	21	2,048		1,898	3,158	2,880	2,126	1,061	0,999	1,484	5,53	UNSATISFACTORY
	22											
LCMSMS SMNE	23	-0,293	-0,129	0,274	-0,335	-1,379	-0,076	-0,625	-0,178	-0,125	-0,96	GOOD

NAB (as received)

	Lab Code	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Performance	
LCMSMS SMNE	1	-0,126	0,585	0,290	0,383	-0,042	1,019	0,261	0,164	-0,216	0,77	GOOD
LCMSMS SMNE	2	-0,473	-0,758	-0,572	-1,206	-0,563	-1,087	-0,536	-0,338	-0,794	-2,11	SATISFACTORY
LCMSMS SMNE	3	-0,352	-0,393	0,912	0,810	-0,149	0,275	0,432		-1,130	0,14	GOOD
LCMSMS SMNE	4							-2,110	-1,944		-2,87	SATISFACTORY
GC TEA	5				1,209	0,599		2,253	2,717	1,596	3,74	QUESTIONABLE
GC NCD	6											
LCMSMS SMNE	7	-0,076	-0,109	-0,382	-0,450	-0,364	0,466	-0,239	-0,092	-0,371	-0,54	GOOD
LCMSMS	8	-0,800	-1,816		-1,510	0,363		0,791	0,122	1,458	-0,53	GOOD
LCMSMS	9	2,636			1,575	2,419		0,164	0,321	1,434	3,49	QUESTIONABLE
LCMSMS	10	0,265	0,753	-1,266	0,184	-0,614	-0,766	0,852	0,482	-0,213	-0,11	GOOD
	11											
GC TEA	12		1,360	0,140	0,713			0,230	-0,088	0,694	1,25	GOOD
LCMSMS SMNE	13	-0,219	0,296	0,615	-0,507	0,102	0,424	-0,363	0,069	-0,430	0,00	GOOD
LCMSMS SMNE	14	-0,279	0,058	-0,001	-0,393	-0,284	0,116	-0,372	-0,336	-0,959	-0,82	GOOD
GC TEA	15											
GC TEA	16							-0,431	-0,324		-0,53	GOOD
LCMSMS SMNE	17	0,629	1,258	1,802	0,607	0,417	1,295	0,415	0,267	0,163	2,28	SATISFACTORY
GC TEA	18											
	19											
	20											
GC TEA	21											
	22											
LCMSMS SMNE	23	-1,205	-1,235	-1,537	-1,414	-1,883	-1,741	-1,347	-1,020	-1,233	-4,20	UNSATISFACTORY

NNK (as received)												
	Lab Code	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**	**Sample7**	**Sample8**	**Sample9**	Performance	
LCMSMS SMNE	**1**	-0,174	-0,101	-1,388	-0,166	0,316	-0,548	-0,098	-0,512	0,366	-0,77	**GOOD**
LCMSMS SMNE	**2**	-1,632	-1,434	-1,685	-1,390	-0,523	-1,339	-1,516	-1,733	-1,323	-4,19	**UNSATISFACTORY**
LCMSMS SMNE	**3**	-0,018	0,753	0,841	0,091	1,335	-0,377	1,096	-0,640	-0,139	0,98	**GOOD**
LCMSMS SMNE	**4**	0,945								-1,555	-0,43	**GOOD**
GC TEA	**5**											
GC NCD	**6**	-0,039		-1,229		-1,157	2,698	0,437	0,373	-1,027	0,02	**GOOD**
LCMSMS SMNE	**7**	-0,314	0,690	0,007	-0,256	0,074	0,597	0,059	0,544	0,592	0,66	**GOOD**
LCMSMS	**8**	-0,244	-0,526	1,460	-0,743	1,795	-0,523	-0,314	0,673	0,685	0,75	**GOOD**
LCMSMS	**9**	-1,386	-1,111	0,090	-1,462	-1,257	-0,438	-1,395	-1,440	-1,027	-3,14	**QUESTIONABLE**
LCMSMS	**10**	0,890	1,399	0,090	1,626	-0,523	0,656	2,043	2,232	1,516	3,31	**QUESTIONABLE**
	11											
GC TEA	**12**	0,739	0,956	1,268	1,604	0,664	0,294	0,433	0,480	2,055	2,83	**SATISFACTORY**
LCMSMS SMNE	**13**	0,257	0,969	0,218	0,870	0,557	0,636	0,495	0,704	0,740	1,82	**GOOD**
LCMSMS SMNE	**14**	-0,547	0,306	1,026	0,189	0,196	-1,087	-0,943	-0,980	-0,148	-0,66	**GOOD**
GC TEA	**15**	1,879				0,354		1,726	0,839	-0,501	1,92	**GOOD**
GC TEA	**16**	-1,439	-1,756	-0,814	-0,649	-0,420	-0,438	-1,134	-1,133	-1,071	-2,95	**SATISFACTORY**
LCMSMS SMNE	**17**	-0,518	0,365	0,552	0,779	0,461	0,360	-0,010	0,454	0,085	0,84	**GOOD**
GC TEA	**18**	0,789				0,362		0,044	-0,004	1,078	1,01	**GOOD**
	19											
	20											
GC TEA	**21**	1,590						-0,283	0,568	-0,029	0,92	**GOOD**
	22											
LCMSMS SMNE	**23**	-0,778	-0,509	-0,437	-0,493	-2,233	-0,492	-0,640	-0,426	-0,299	-2,10	**SATISFACTORY**

NNN (corrected)												
	Lab Code	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**	**Sample7**	**Sample8**	**Sample9**	Performance	
LCMSMS SMNE	**1**	0,038	0,198	0,017	0,270	-0,196	0,205	0,011	0,074	0,097	0,24	**GOOD**
LCMSMS SMNE	**2**	-0,649	-0,526	-0,215	-1,240	-0,571	-0,633	-0,277	-0,253	-0,632	-1,67	**GOOD**
LCMSMS SMNE	**3**	0,556	0,222	1,078	0,202	0,317	0,319	0,063	0,372	-0,345	0,93	**GOOD**
LCMSMS SMNE	**4**	-1,962		-1,742				-2,295	-1,727	-2,092	-4,39	**UNSATISFACTORY**
GC TEA	**5**											
GC NCD	**6**	0,384	0,701	-0,155	0,146	-0,249	0,440	1,394	0,985	0,790	1,48	**GOOD**
LCMSMS SMNE	**7**	0,575	0,878	0,815	1,145	0,551	0,627	0,618	1,151	1,017	2,46	**SATISFACTORY**
LCMSMS	**8**	0,918	-2,013	-0,845	-0,620	1,986		0,177		0,061	-0,13	**GOOD**
LCMSMS	**9**	0,065	0,595	-0,007	-0,752	-0,577	-0,182	-0,165	0,053	-0,056	-0,34	**GOOD**
LCMSMS	**10**	-0,590	-0,667	-1,254	-0,243	-1,040	-1,097	-0,052	0,240	0,247	-1,49	**GOOD**
	11											
GC TEA	**12**	0,873	0,766	0,018	0,764	0,526	0,245	0,500	0,626	1,188	1,84	**GOOD**
LCMSMS SMNE	**13**	1,339	0,748	1,800	0,768	0,384	0,991	1,507		0,943	3,00	**SATISFACTORY**
LCMSMS SMNE	**14**	0,227	0,755	0,485	0,997	0,478	0,442	0,142	0,282	0,315	1,37	**GOOD**
GC TEA	**15**											
GC TEA	**16**											
LCMSMS SMNE	**17**	0,165	0,273	0,636	1,290	0,026	0,820	0,482	0,831	0,465	1,66	**GOOD**
GC TEA	**18**											
	19											
	20											
GC TEA	**21**	0,301		0,846	-0,508	0,734	0,547	-0,253	-0,364	0,174	0,52	**GOOD**
	22											
LCMSMS SMNE	**23**	-2,241	-1,931	-1,477	-2,218	-2,370	-2,725	-1,851	-2,271	-2,173	-6,42	**UNSATISFACTORY**

NAT (corrected)												
	Lab Code	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**	**Sample7**	**Sample8**	**Sample9**	Performance	
LCMSMS SMNE	**1**	-1,050	-0,513	-0,252	-0,486	-0,824	-0,380	-0,347	-0,662	-0,165	-1,56	**GOOD**
LCMSMS SMNE	**2**	-0,805	-0,379	0,173	-0,624	-0,362	0,019	-0,388	-0,376	-0,804	-1,18	**GOOD**
LCMSMS SMNE	**3**	-0,031	0,980	-0,044	-0,571	0,107	0,259	-0,178		0,137	0,23	**GOOD**
LCMSMS SMNE	**4**	0,813		-2,442				-0,699	-0,899	-1,614	-2,17	**SATISFACTORY**
GC TEA	**5**											
GC NCD	**6**	0,110	-0,404	-0,912	-1,035	-0,024	-0,239	1,355	0,744	0,438	0,01	**GOOD**
LCMSMS SMNE	**7**	0,317	0,464	0,571	0,232	0,199	0,512	0,694	0,578	1,069	1,54	**GOOD**
LCMSMS	**8**	0,709	-1,688	-0,062	-0,750	0,016	-2,512	-0,179	-0,061	0,274	-1,42	**GOOD**
LCMSMS	**9**	0,299	0,790	0,548	0,297	-0,191	0,458	-0,290	0,126	-0,157	0,63	**GOOD**
LCMSMS	**10**	-1,138	-0,640	-0,968	-0,414	-1,185	-0,514	-0,187	-0,062	-0,024	-1,71	**GOOD**
	11											
GC TEA	**12**		0,652	-0,110	0,656	1,317	0,035	0,735	0,519		1,44	**GOOD**
LCMSMS SMNE	**13**	1,256	1,287	1,511	0,419	0,981	1,116	1,502	1,833	1,572	3,83	**QUESTIONABLE**
LCMSMS SMNE	**14**	0,337	0,133	0,521	-0,051	0,060	0,643	0,132	0,168	0,202	0,72	**GOOD**
GC TEA	**15**											
GC TEA	**16**											
LCMSMS SMNE	**17**	1,057	1,079	1,356	1,062	0,547	1,050	1,016	1,114	1,173	3,15	**QUESTIONABLE**
GC TEA	**18**											
	19											
	20											
GC TEA	**21**	0,480		0,808	2,577	1,616	0,964	-0,614	-0,728	-0,008	1,80	**GOOD**
	22											
LCMSMS SMNE	**23**	-2,355	-1,763	-0,697	-1,313	-2,256	-1,412	-2,552	-2,296	-2,093	-5,58	**UNSATISFACTORY**

NAB (corrected)												
	Lab Code	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**	**Sample7**	**Sample8**	**Sample9**	Performance	
LCMSMS SMNE	**1**	-0,669	-0,317	-0,574	-0,422	-0,433	-0,082	-0,628	-0,497	-0,667	-1,43	**GOOD**
LCMSMS SMNE	**2**	-0,375	-0,591	-0,416	-0,937	-0,447	-0,692	-0,409	-0,260	-0,612	-1,58	**GOOD**
LCMSMS SMNE	**3**	-0,615	-0,695	0,077	0,156	-0,360	-0,232	-0,151		-1,138	-1,05	**GOOD**
LCMSMS SMNE	**4**							-1,860	-2,112		-2,81	**SATISFACTORY**
GC TEA	**5**											
GC NCD	**6**											
LCMSMS SMNE	**7**	0,323	0,325	0,190	0,115	-0,028	0,925	0,460	0,557	0,091	0,99	**GOOD**
LCMSMS	**8**	-0,490	-1,240		-1,039	0,417		1,024	0,472	1,410	0,21	**GOOD**
LCMSMS	**9**	2,462			1,778	2,353		0,736	0,965	1,620	4,05	**UNSATISFACTORY**
LCMSMS	**10**	-0,182	0,074	-1,224	-0,267	-0,685	-0,855	0,210	0,147	-0,454	-1,08	**GOOD**
	11											
GC TEA	**12**		1,779	0,828	1,404			1,231	0,822	1,268	2,99	**SATISFACTORY**
LCMSMS SMNE	**13**	0,148	0,583	0,856	0,001	0,343	0,815	0,254	0,679	-0,013	1,22	**GOOD**
LCMSMS SMNE	**14**	0,101	0,396	0,405	0,102	0,007	0,586	0,248	0,183	-0,480	0,52	**GOOD**
GC TEA	**15**											
GC TEA	**16**											
LCMSMS SMNE	**17**	0,725	1,204	1,566	0,824	0,535	1,310	0,829	0,771	0,408	2,72	**SATISFACTORY**
GC TEA	**18**											
	19											
	20											
GC TEA	**21**											
	22											
LCMSMS SMNE	**23**	-1,427	-1,519	-1,709	-1,713	-1,703	-1,774	-1,945	-1,726	-1,433	-4,98	**UNSATISFACTORY**

NNK (corrected)

	Lab Code	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	Sample9	Performance	
LCMSMS SMNE	1	-0,331	-0,557	-1,296	-0,509	-0,116	-0,657	-0,642	-0,845	-0,283	-1,75	GOOD
LCMSMS SMNE	2	-1,097	-1,296	-1,212	-1,009	-0,455	-0,764	-0,965	-1,105	-0,755	-2,89	SATISFACTORY
LCMSMS SMNE	3	-0,071	0,256	0,170	-0,205	0,703	-0,483	-0,055	-0,777	-0,406	-0,29	GOOD
LCMSMS SMNE	4	-1,015						0,828	1,111	-1,738	-0,41	GOOD
GC TEA	5											
GC NCD	6	1,752		0,122		-0,143	2,629	0,990	1,656	0,196	2,72	SATISFACTORY
LCMSMS SMNE	7	-0,010	0,505	-0,106	-0,175	0,001	0,199	-0,274	0,158	0,090	0,13	GOOD
LCMSMS	8	0,299	-0,300	1,076	-0,309	1,500	-0,180	-0,263	0,502	0,297	0,87	GOOD
LCMSMS	9	-0,056	-0,250	0,743	-0,291	-0,503	0,357	-0,312	-0,052	-0,091	-0,15	GOOD
LCMSMS	10	-0,253	-0,093	-0,928	-0,024	-1,075	-0,598	-0,318	-0,223	-0,272	-1,26	GOOD
	11											
GC TEA	12	1,252	1,112	1,049	1,578	0,703	0,319	0,152	0,509	1,001	2,56	SATISFACTORY
LCMSMS SMNE	13	1,112	1,398	0,546	1,287	0,804	0,695	0,371	0,920	0,566	2,57	SATISFACTORY
LCMSMS SMNE	14	0,418	0,808	1,142	0,770	0,525	-0,219	-0,296	-0,070	0,154	1,08	GOOD
GC TEA	15											
GC TEA	16											
LCMSMS SMNE	17	0,107	0,520	0,501	0,878	0,495	0,303	-0,097	0,422	0,051	1,06	GOOD
GC TEA	18											
	19											
	20											
GC TEA	21							2,676		2,581	3,72	QUESTIONABLE
	22											
LCMSMS SMNE	23	-2,106	-2,102	-1,808	-1,991	-2,438	-1,600	-1,795	-2,206	-1,392	-5,81	UNSATISFACTORY

Water after												
	Lab Code	**Sample1**	**Sample2**	**Sample3**	**Sample4**	**Sample5**	**Sample6**	**Sample7**	**Sample8**	**Sample9**	Performance	
GC CRM 57	**1**	0,808	0,881	0,607	1,935	1,726	0,689	0,610	0,642	0,500	2,80	**SATISFACTORY**
KF OTHER	**2**	-0,343	-1,195	-0,518	-1,091	-2,340	-0,591	-0,975	0,000	-0,304	-2,45	**SATISFACTORY**
GC OTHER	**3**	-1,855	-1,511	-2,931	-1,357	-1,067	-2,523	-0,984	-0,607	-1,180	-4,67	**UNSATISFACTORY**
KF CRM 56	**4**											
	5											
KF OTHER	**6**	0,101	-0,787	1,409	-0,186	0,586	0,472		-0,338		0,47	**GOOD**
GC CRM 57	**7**	-0,298	0,268	1,131	0,746	0,369	1,226	0,238	0,284	-0,211	1,25	**GOOD**
GC CRM 57	**8**	-0,046	0,307	0,031	1,669	1,447	-0,021	0,978	0,406	0,065	1,61	**GOOD**
GC CRM 57	**9**	0,823		-0,827	0,094	-1,221	-1,873	-2,624	-2,720	-2,486	-3,83	**QUESTIONABLE**
KF CRM 56	**10**											
KF OTHER	**11**	1,232	0,710	0,109	0,906	0,521	0,648	0,372	0,431	1,060	2,00	**GOOD**
GC CRM 57	**12**	-0,792	-0,024	1,497	0,227	-0,334	-0,200	0,198	0,018	-0,413	0,06	**GOOD**
KF OTHER	**13**	0,353	0,502	-0,055	0,502	-0,002	0,106	0,183	0,263	0,419	0,76	**GOOD**
KF OTHER	**14**	-1,283	-0,385	-0,441	-0,966	-0,234	-0,673	-0,221	-0,353	-0,461	-1,67	**GOOD**
GC OTHER	**15**	-0,489	-0,029	-0,474	-0,719	-0,513	-0,244	-0,440		0,061	-1,01	**GOOD**
KF CRM 56	**16**	0,427	0,808	0,217	-0,026	0,412	0,729	0,535	0,561	0,407	1,36	**GOOD**
KF OTHER	**17**		0,105	0,194		0,461	0,380	0,446			0,71	**GOOD**
KF OTHER	**18**	2,185	2,417	1,141	0,540	1,099	1,623	1,984	2,268	1,780	5,01	**UNSATISFACTORY**
KF CRM 56	**19**	0,400	-0,141	-0,026	-0,113	0,274	0,304		0,279	1,303	0,81	**GOOD**
KF CRM 56	**20**	-1,595	-1,997	-0,809	0,260	0,193	-0,897	-0,848	-1,173	-1,081	-2,65	**SATISFACTORY**
	21											
KF OTHER	**22**	-0,065	-0,356	-0,314	-0,516	0,015	0,302	0,094	-0,271	0,310	-0,27	**GOOD**
NIR transmittance	**23**	0,438	0,427	0,059	-1,905	-1,392	0,543	0,453	0,310	0,232	-0,28	**GOOD**

APPENDIX:

Mean and standard deviations derived from the raw data.

Water before (%)

MEAN		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
GC CRM 57	1	18,59	51,77	18,29	5,73	4,46	24,56	52,97	48,76	29,33
KF OTHER	2	18,51	49,29	16,81	2,79	2,85	23,73	52,16	49,05	29,79
GC OTHER	3	16,53	48,79	12,50	2,88	3,11	19,00	47,42	46,00	26,15
KF CRM 56	4									
	5									
	6									
GC CRM 57	7	17,75	52,79	19,90	4,17	4,04	25,84	52,02	48,16	27,17
GC CRM 57	8	18,22	54,06	18,09	5,39	4,55	23,70	54,34	48,51	28,58
GC CRM 57	9	18,30	43,99	18,37	5,30	4,58	23,34	39,67	38,95	26,35
KF CRM 56	10	18,55	52,52	17,17	3,89	3,63	24,72	54,16	48,97	31,58
KF OTHER	11	21,38	54,63	18,05	5,26	5,57	25,20	54,69	51,81	31,92
GC CRM 57	12	17,12	52,08	20,64	4,19	3,83	24,10	52,34	47,31	28,47
KF OTHER	13	19,86	53,41	17,90	3,93	3,57	24,31	53,46	48,76	30,12
KF OTHER	14	18,27	52,28	18,45	3,31	2,21	22,41	51,72	47,70	30,60
	15									
KF CRM 56	16	18,95	53,30	18,28	4,42	4,20	24,85	54,19	49,83	29,69
KF OTHER	17	18,84	52,75	18,30	4,92	4,52	24,85	53,27	49,65	28,60
KF OTHER	18	20,27	56,67	19,10	4,57	4,57	27,10	56,77	53,50	32,10
KF CRM 56	19	19,16	52,99	18,29	4,37	4,29	24,83	53,60	50,18	29,48
KF CRM 56	20	17,04	47,46	16,32	4,12	3,96	21,76	47,53	43,79	26,32
	21									
KF OTHER	22	18,48	51,55	16,69	4,10	4,01	23,65	51,63	47,29	29,29
NIR transmittance	23	18,88	53,05	17,87	2,82	3,45	24,68	53,24	49,04	29,63

STANDARD DEVIATION		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
GC CRM 57	1	0,039	0,099	0,056	0,019	0,043	0,108	0,107	0,240	0,286
KF OTHER	2	0,434	1,923	0,321	0,460	0,135	0,066	0,151	0,046	0,673
GC OTHER	3	0,114	0,278	0,144	0,196	0,107	0,177	0,330	0,239	0,078
KF CRM 56	4									
	5									
KF OTHER	6									
GC CRM 57	7	0,188	0,685	0,097	0,038	0,031	0,117	0,259	0,198	0,466
GC CRM 57	8	0,261	0,880	0,275	0,036	0,056	0,336	0,694	1,506	0,396
GC CRM 57	9	0,032	2,448	0,085	0,040	0,064	0,312	0,310	0,179	0,144
KF CRM 56	10	0,035	0,262	0,586	0,488	0,246	0,144	0,960	0,338	0,389
KF OTHER	11	0,335	1,136	0,162	0,170	0,000	0,450	0,127	0,975	0,290
GC CRM 57	12	0,108	0,321	0,670	0,016	0,027	0,362	0,417	1,153	0,021
KF OTHER	13	1,123	0,562	0,314	0,140	0,262	0,323	0,135	0,159	0,413
KF OTHER	14	0,058	0,125	0,038	0,138	0,058	0,658	0,149	0,191	0,391
GC OTHER	15									
KF CRM 56	16	0,087	0,354	0,240	0,041	0,101	0,108	0,554	0,117	0,391
KF OTHER	17	0,262	1,032	0,184	0,615	0,721	0,679	0,035	0,544	0,007
KF OTHER	18	0,231	0,666	0,500	0,115	0,058	0,100	0,987	0,693	0,800
KF CRM 56	19	0,025	0,296	0,078	0,077	0,102	0,037	0,429	0,531	0,095
KF CRM 56	20	0,145	0,424	0,540	0,075	0,157	0,170	0,568	0,364	0,552
	21									
KF OTHER	22	0,132	0,161	0,795	0,047	0,050	0,456	0,014	0,158	0,230
NIR transmittance	23	0,290	0,104	0,099	0,098	0,285	0,030	0,046	0,169	0,585

Moisture

MEAN		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
	1	24,43	58,60	22,76	5,69	5,02	26,49	55,54	52,79	32,60
	2	24,16	57,60	19,80	4,76	5,35	24,54	55,39	52,34	32,49
	3	24,45	57,76	19,15	4,52	4,25	24,91	54,79	51,92	30,68
	4	23,33	58,52	21,70	5,65	4,69	26,20	55,49	52,46	32,06
	5	24,99	58,94	25,19	6,04	5,40	28,27	56,08	53,87	33,45
	6	24,21	58,88	22,32	5,61	4,77	26,19	55,84	52,65	32,65
	7	22,68	57,40	21,32	5,49	4,79	26,20	55,42	52,78	31,39
	8	24,91	58,84	22,02	5,83	4,84	26,32	55,55	53,05	32,50
	9	24,10	59,03	23,07	5,80	5,23	26,87	55,63	52,67	31,53
	10	27,28	60,20	25,77	6,53	5,49	28,87	56,19	53,67	33,56
	12	23,90	58,63	21,65	5,39	4,65	25,99	55,12	52,86	32,27
	13	24,32	58,48	21,84	5,50	4,70	26,04	55,32	52,57	31,88
	14	24,60	59,14	23,40	6,12	5,01	26,92	56,05	52,90	32,54
	15	23,95	58,40	22,06	5,05	4,29	26,20	54,49	51,99	31,39
	17	23,40	57,55	21,42	4,82	4,62	24,82	54,84	51,89	30,82
	19	23,12	56,95	18,91	4,02	4,50	24,10	55,10	51,53	32,02
	20	23,80	58,30	21,29	5,27	4,13	25,54	55,16	52,29	31,37
	21	23,43	57,47	21,76	4,85	4,82	25,35	54,82	52,47	31,15
	22	25,38	59,39	24,86	5,93	5,32	28,64	55,77	53,12	33,94
	23	24,28	58,36	22,92	5,87	5,02	27,31	56,06	53,40	32,88

STANDARD DEVIATION		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
	1	0,136	0,428	0,243	0,014	0,031	0,099	0,147	0,219	0,106
	2	0,310	0,176	0,430	0,266	0,165	0,215	0,079	0,595	0,446
	3	0,644	0,250	0,144	0,157	0,261	0,090	0,117	0,128	0,396
	4	0,093	0,063	0,263	0,036	0,020	0,220	0,135	0,109	0,193
	5	0,706	0,602	0,512	0,180	0,105	0,268	0,145	0,024	0,241
	6	0,119	0,144	0,184	0,062	0,044	0,078	0,067	0,174	0,263
	7	0,069	0,275	0,160	0,010	0,007	0,019	0,002	0,067	0,122
	8	0,205	0,163	0,044	0,098	0,029	0,018	0,066	0,050	0,030
	9	0,173	0,058	0,153	0,100	0,058	0,115	0,058	0,153	0,252
	10	0,413	0,100	0,346	0,044	0,091	0,264	0,070	0,222	0,071
	12	0,045	0,036	0,173	0,052	0,027	0,036	0,057	0,583	0,069
	13	0,162	0,371	0,055	0,059	0,028	0,038	0,058	0,102	0,041
	14	0,141	0,046	0,012	0,019	0,023	0,107	0,029	0,035	0,042
	15	0,185	0,095	0,235	0,090	0,061	0,145	0,089	0,173	0,596
	17	0,155	0,511	0,113	0,153	0,058	0,304	0,060	0,195	0,038
	19	0,251	0,266	0,201	0,015	0,206	0,244	0,030	0,210	0,070
	20	0,800	0,301	0,337	0,231	0,115	0,423	0,280	0,351	0,831
	21	0,435	0,483	0,729	0,045	0,230	0,573	0,053	0,123	0,288
	22	0,179	0,096	0,209	0,036	0,028	0,240	0,202	0,131	0,180
	23	0,186	0,622	0,025	0,036	0,064	0,420	0,215	0,079	0,095

pH

MEAN		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
CDC	**1**	8,11	8,45	5,29	8,73	7,51	5,93	7,73	8,26	7,41
CDC?	**2**	7,98	8,04	5,24	8,76	7,48	5,93	7,61	8,10	7,38
CDC	**3**	8,18	8,33	5,24	8,58	7,64	6,15	7,71	7,04	6,39
CDC	**4**	8,10	8,57	5,34	8,64	7,67	6,09	7,86	8,40	7,87
CDC	**5**	7,89	8,50	5,28	8,63	7,57	6,03	7,64	8,12	7,38
OTHER	**6**	7,93	8,51	5,23	8,74	7,79	6,08	7,73	8,22	7,49
CDC	**7**	8,09	8,37	5,23	8,43	7,67	5,96	7,72	8,30	7,50
CDC	**8**	8,25	8,53	5,43	8,88	7,76	6,03	7,99	8,65	7,59
CDC	**9**	8,20	8,56	5,30	8,69	7,78	6,09	7,91	8,40	7,50
CDC?	**10**	8,14	8,55	5,22	8,67	7,52	5,92	7,74	8,27	7,43
CDC	**12**	8,04	8,22	5,24	8,51	7,45	5,87	7,88	8,23	7,44
CDC?	**13**	8,02	8,31	5,23	8,38	7,56	6,01	7,73	8,23	7,40
CDC	**14**	7,81	8,19	5,14	8,50	7,46	5,91	7,69	8,19	7,15
CDC?	**15**	7,95	8,24	5,19	8,83	7,48	5,93	7,62	8,17	7,38
CDC?	**16**	7,98	8,25	5,25	8,39	7,57	5,99	7,65	8,20	7,38
CDC?	**18**	8,19	7,39	5,14	8,40	8,07	5,89	7,39	8,15	7,61
CDC	**19**	8,06	8,43	5,25	8,69	7,57	6,01	7,68	8,18	7,37
CDC?	**20**	8,02	8,41	5,30	8,65	7,71	6,08	7,68	8,20	7,43
CDC	**21**	8,06	8,58	5,26	8,81	7,61	5,98	7,74	8,21	7,30
CDC?	**22**	8,21	8,37	5,33	8,91	7,68	5,99	7,75	8,38	7,58
CDC	**23**	8,01	8,38	5,20	8,61	7,56	5,93	7,74	8,28	7,39

STANDARD DEVIATION		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
CDC	**1**	0,004	0,035	0,031	0,034	0,046	0,025	0,040	0,033	0,042
CDC?	**2**	0,007	0,008	0,003	0,023	0,042	0,003	0,005	0,004	0,007
CDC	**3**	0,035	0,015	0,055	0,035	0,031	0,017	0,047	0,038	0,036
CDC	**4**	0,006	0,025	0,017	0,020	0,055	0,006	0,000	0,017	0,684
CDC	**5**	0,015	0,050	0,012	0,010	0,012	0,006	0,031	0,015	0,012
OTHER	**6**	0,013	0,028	0,011	0,019	0,005	0,005	0,009	0,007	0,015
CDC	**7**	0,000	0,006	0,006	0,006	0,010	0,006	0,006	0,006	0,006
CDC	**8**	0,022	0,039	0,003	0,010	0,028	0,008	0,006	0,010	0,006
CDC	**9**	0,006	0,065	0,006	0,006	0,031	0,015	0,025	0,000	0,012
CDC?	**10**	0,006	0,017	0,010	0,026	0,020	0,035	0,006	0,006	0,017
CDC	**12**	0,023	0,060	0,012	0,166	0,021	0,006	0,042	0,021	0,006
CDC?	**13**	0,003	0,013	0,000	0,005	0,005	0,006	0,003	0,005	0,006
CDC	**14**	0,010	0,012	0,010	0,006	0,026	0,006	0,017	0,000	0,015
CDC?	**15**	0,010	0,035	0,010	0,045	0,000	0,017	0,012	0,023	0,000
CDC?	**16**	0,007	0,046	0,025	0,073	0,083	0,027	0,012	0,018	0,009
CDC?	**18**	0,137	0,199	0,023	0,015	0,140	0,044	0,157	0,071	0,092
CDC	**19**	0,067	0,130	0,021	0,023	0,025	0,046	0,078	0,133	0,036
CDC?	**20**	0,072	0,155	0,012	0,036	0,020	0,026	0,026	0,087	0,020
CDC	**21**	0,012	0,031	0,006	0,006	0,012	0,012	0,000	0,015	0,015
CDC?	**22**	0,018	0,033	0,065	0,033	0,107	0,043	0,021	0,007	0,009
CDC	**23**	0,026	0,022	0,002	0,034	0,004	0,005	0,015	0,016	0,017

Nicotine (%) as is

MEAN		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
CRM62 HEX	1	0,68	0,74	2,16	0,23	0,41	0,81	1,15	1,17	1,33
?	2	0,65	0,73	1,94	0,25	0,36	0,81	1,15	1,17	1,50
OWN	3	0,70	0,76	2,15	0,22	0,43	0,78	1,22	1,24	1,42
CDC	4	0,37	0,76	1,59	0,21	0,31	0,66	0,87	0,40	1,04
?	5	0,67	0,73	1,91	0,23	0,39	0,82	1,17	1,21	1,48
CDC	6	0,77	0,82	2,40	0,32	0,48	0,90	1,26	1,31	1,09
CDC?	7	0,70	0,75	2,25	0,22	0,40	0,81	1,18	1,23	1,51
CDC	8	0,71	0,75	2,35	0,22	0,40	0,84	1,23	1,28	1,55
CRM62 MTBE	9	0,76	0,82	2,40	0,28	0,45	0,89	1,20	1,22	1,50
OWN	10	0,74	0,82	2,35	0,23	0,42	0,79	1,19	1,22	1,57
OWN	11	0,72	0,74	2,32	0,23	0,40	0,82	1,19	1,19	1,50
CDC	12	0,67	0,72	2,08	0,27	0,41	0,79	1,12	1,14	1,35
CDC	13	0,70	0,74	2,32	0,25	0,39	0,83	1,18	1,22	1,50
CRM62	14	0,76	0,80	2,32	0,30	0,33	0,90	1,26	1,26	1,52
OWN TNA?	15	0,82	0,87	2,19	0,34	0,54	0,95	1,31	1,33	1,55
CRM62 MTBE	16	1,04	1,10	3,03	0,34	0,59	1,17	1,71	1,70	2,03
OWN	17	0,74	0,77	2,27	0,24	0,46	0,83	1,22	1,25	1,54
OWN	18	0,89	0,92	2,86	0,24	0,49	1,02	1,48	1,47	1,86
OWN	19	0,78	0,76	2,33	0,26	0,41	0,97	1,22	1,23	1,55
CRM62 HEX MOD	20	0,63	0,67	1,94	0,22	0,36	0,73	1,06	1,10	1,34
CDC?	21	0,69	0,73	2,35	0,25	0,40	0,87	1,21	1,24	1,71
OWN	22	0,62	0,79	2,29	0,23	0,41	0,89	1,32	1,19	1,56
OWN	23	0,79	0,80	1,98	0,21	0,27	0,62	1,25	1,40	1,61

STANDARD DEVIATION		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
CRM62 HEX	1	0,004	0,002	0,008	0,005	0,008	0,003	0,005	0,004	0,163
?	2	0,003	0,003	0,020	0,011	0,050	0,011	0,001	0,022	0,003
OWN	3	0,010	0,012	0,063	0,011	0,008	0,019	0,006	0,010	0,074
CDC	4	0,238	0,050	0,195	0,020	0,016	0,086	0,096	0,225	0,104
?	5	0,031	0,001	0,080	0,021	0,003	0,002	0,012	0,014	0,012
CDC	6	0,004	0,005	0,040	0,012	0,002	0,010	0,007	0,031	0,059
CDC?	7	0,012	0,012	0,066	0,005	0,002	0,017	0,019	0,003	0,027
CDC	8	0,003	0,003	0,028	0,017	0,003	0,002	0,002	0,004	0,007
CRM62 MTBE	9	0,032	0,005	0,026	0,013	0,004	0,004	0,011	0,027	0,060
OWN	10	0,012	0,004	0,057	0,017	0,008	0,002	0,011	0,010	0,040
OWN	11	0,017	0,000	0,021	0,010	0,000	0,006	0,000	0,012	0,010
CDC	12	0,006	0,007	0,032	0,004	0,003	0,003	0,006	0,005	0,016
CDC	13	0,003	0,003	0,020	0,004	0,012	0,005	0,001	0,017	0,005
CRM62	14	0,000	0,006	0,000	0,006	0,000	0,035	0,015	0,006	0,000
OWN TNA?	15	0,029	0,015	0,078	0,021	0,021	0,012	0,000	0,012	0,010
CRM62 MTBE	16	0,017	0,022	0,121	0,006	0,009	0,034	0,038	0,031	0,077
OWN	17	0,030	0,007	0,040	0,010	0,005	0,010	0,009	0,013	0,029
OWN	18	0,020	0,006	0,029	0,020	0,005	0,008	0,013	0,014	0,047
OWN	19	0,020	0,042	0,096	0,022	0,015	0,168	0,024	0,053	0,124
CRM62 HEX MOD	20	0,015	0,006	0,121	0,016	0,003	0,014	0,024	0,040	0,023
CDC?	21	0,012	0,007	0,075	0,020	0,006	0,012	0,024	0,030	0,032
OWN	22	0,039	0,037	0,034	0,012	0,017	0,013	0,015	0,069	0,091
OWN	23	0,042	0,035	0,028	0,015	0,010	0,041	0,009	0,041	0,054

TSNA sum

MEAN		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
LCMSMS SMNE	**1**	2,34	0,62	0,95	0,84	0,65	0,94	5,47	6,69	1,67
LCMSMS SMNE	**2**	2,07	0,54	0,92	0,67	0,60	0,86	4,99	6,30	1,41
LCMSMS SMNE	**3**	2,41	0,65	1,05	0,80	0,75	0,93	5,34	6,45	1,53
LCMSMS SMNE	**4**	2,21		0,48				4,60	5,92	1,17
GC TEA	**5**	1,22	0,24	0,51	0,39	0,27	0,46	3,19	4,15	0,90
GC NCD	**6**	2,15	0,56	0,71	0,61	0,55	0,83	5,69	6,68	1,48
LCMSMS SMNE	**7**	2,41	0,66	1,06	0,86	0,71	0,96	5,75	7,34	1,78
LCMSMS	**8**	2,57	0,44	0,85	0,71	0,87	0,58	5,40	6,70	1,67
LCMSMS	**9**	2,14	0,61	0,92	0,70	0,55	0,86	4,61	6,00	1,43
LCMSMS	**10**	2,58	0,67	0,86	0,95	0,59	0,99	6,41	8,18	1,97
GC TEA	**12**	2,56	0,63	0,87	0,87	0,72	0,87	5,34	6,51	1,75
LCMSMS SMNE	**13**	2,51	0,65	1,16	0,83	0,72	0,95	5,90	7,39	1,69
LCMSMS SMNE	**14**	2,04	0,56	0,88	0,82	0,61	0,80	4,21	5,01	1,34
GC TEA	**15**	2,59	0,42	1,05	0,56	0,68	0,89	5,10	6,30	1,41
GC TEA	**16**	2,12	0,52	0,96	1,01	0,56	0,93	5,47	6,93	1,51
LCMSMS SMNE	**17**	2,32	0,63	1,07	0,89	0,70	0,96	5,61	7,23	1,66
GC TEA	**18**	2,48	0,63	1,15	0,68	0,68	1,04	5,49	5,86	1,70
GC TEA	**21**	2,86	1,24	1,20	1,17	0,65	1,05	5,92	7,41	1,82
LCMSMS SMNE	**23**	2,09	0,54	0,92	0,72	0,37	0,84	4,58	5,93	1,44

STANDARD DEVIATION		Sample								
Method	LAB Code	1	2	3	4	5	6	7	8	9
LCMSMS SMNE	**1**	0,027	0,009	0,089	0,048	0,010	0,048	0,090	0,118	0,030
LCMSMS SMNE	**2**	0,079	0,008	0,081	0,023	0,010	0,045	0,016	0,087	0,022
LCMSMS SMNE	**3**	0,231	0,019	0,060	0,027	0,072	0,014	0,057	0,479	0,135
LCMSMS SMNE	**4**	0,021		0,032				0,077	0,122	0,079
GC TEA	**5**	0,012	0,033	0,046	0,007	0,011	0,014	0,059	0,029	0,009
GC NCD	**6**	0,094	0,029	0,096	0,034	0,046	0,034	0,069	0,150	0,020
LCMSMS SMNE	**7**	0,030	0,000	0,036	0,018	0,006	0,024	0,073	0,279	0,005
LCMSMS	**8**	0,121	0,060	0,075	0,056	0,007	0,219	0,120	0,312	0,036
LCMSMS	**9**	0,060	0,017	0,050	0,023	0,017	0,043	0,270	0,058	0,057
LCMSMS	**10**	0,030	0,015	0,090	0,016	0,087	0,097	0,081	0,052	0,033
GC TEA	**12**	0,421	0,003	0,020	0,010	0,029	0,031	0,024	0,129	0,013
LCMSMS SMNE	**13**	0,033	0,005	0,088	0,062	0,013	0,028	0,160	0,255	0,062
LCMSMS SMNE	**14**	0,009	0,013	0,015	0,015	0,010	0,016	0,059	0,098	0,016
GC TEA	**15**	0,056	0,156	0,017	0,109	0,036	0,039	0,125	0,151	0,129
GC TEA	**16**	0,110	0,024	0,125	0,199	0,012	0,028	0,249	0,174	0,047
LCMSMS SMNE	**17**	0,025	0,007	0,077	0,042	0,031	0,016	0,036	0,087	0,023
GC TEA	**18**	0,079	0,011	0,053	0,020	0,033	0,015	0,240	0,216	0,053
GC TEA	**21**	0,104	0,346	0,067	0,019	0,003	0,007	0,019	0,070	0,019
LCMSMS SMNE	**23**	0,021	0,005	0,026	0,024	0,109	0,011	0,098	0,077	0,038

Water after (%)

MEAN		Sample								
Method	**LAB Code**	1	2	3	4	5	6	7	8	9
GC CRM 57	**1**	19,12	53,85	18,70	5,63	4,98	24,98	53,87	49,96	30,02
KF OTHER	**2**	17,96	49,27	16,67	3,17	2,73	22,57	47,22	47,69	28,03
GC OTHER	**3**	16,45	48,57	12,32	2,96	3,43	18,94	47,18	45,54	25,86
KF CRM 56	**4**									
KF OTHER	**6**	18,41	50,17	20,15	3,91	4,35	24,57	50,99	46,49	27,75
GC CRM 57	**7**	18,01	52,50	19,65	4,67	4,23	25,99	52,31	48,69	28,26
GC CRM 57	**8**	18,26	52,58	17,66	5,42	4,83	23,64	55,41	49,12	28,95
GC CRM 57	**9**	19,13	45,11	16,11	4,14	3,35	20,16	40,31	38,08	22,62
KF CRM 56	**10**									
KF OTHER	**11**	19,54	53,47	17,80	4,80	4,31	24,90	52,87	49,21	31,41
GC CRM 57	**12**	17,51	51,85	20,31	4,25	3,84	23,31	52,14	47,75	27,76
KF OTHER	**13**	18,66	53,01	17,51	4,47	4,02	23,88	52,08	48,62	29,83
KF OTHER	**14**	17,02	51,06	16,81	3,28	3,90	22,42	50,38	46,44	27,64
GC OTHER	**15**	17,82	51,84	16,75	3,48	3,74	23,22	49,47	47,89	28,94
KF CRM 56	**16**	18,74	53,69	18,00	4,04	4,25	25,05	53,55	49,67	29,80
KF OTHER	**17**	18,48	52,14	17,96	4,97	4,28	24,40	53,18	49,38	29,61
KF OTHER	**18**	20,50	57,23	19,67	4,50	4,63	26,73	59,63	55,70	33,20
KF CRM 56	**19**	18,71	51,59	17,56	3,97	4,18	24,25	51,86	48,67	32,02
KF CRM 56	**20**	16,71	47,50	16,15	4,27	4,13	21,99	47,76	43,54	26,11
KF OTHER	**22**	18,24	51,12	17,04	3,64	4,03	24,25	51,70	46,73	29,55
NIR transmittance	**23**	18,75	52,85	17,71	2,51	3,25	24,70	53,21	48,78	29,36

STANDARD DEVIATION		Sample								
Method	**LAB Code**	1	2	3	4	5	6	7	8	9
GC CRM 57	**1**	0,069	0,084	0,099	0,321	0,095	0,033	0,272	0,202	0,198
KF OTHER	**2**	0,361	1,329	0,161	0,040	0,224	0,041	0,192	0,149	1,088
GC OTHER	**3**	0,070	0,019	0,288	0,070	0,042	0,072	0,055	0,038	0,201
KF CRM 56	**4**									
KF OTHER	**6**	0,408	1,512	0,097	0,127	0,145	0,075	2,114	1,835	1,390
GC CRM 57	**7**	0,094	0,500	0,144	0,039	0,033	0,187	0,111	0,220	0,386
GC CRM 57	**8**	0,125	0,875	0,281	0,019	0,034	0,441	0,426	0,337	0,148
GC CRM 57	**9**	0,250	4,525	0,825	0,097	0,011	0,092	0,205	0,103	0,376
KF CRM 56	**10**									
KF OTHER	**11**	0,108	0,725	0,228	0,092	0,153	0,191	0,139	0,459	0,276
GC CRM 57	**12**	0,068	0,397	0,390	0,196	0,247	0,066	0,252	0,350	0,095
KF OTHER	**13**	0,264	0,376	0,153	0,258	0,124	0,474	0,280	0,140	0,773
KF OTHER	**14**	0,085	0,132	0,161	0,088	0,066	0,380	0,482	0,099	0,342
GC OTHER	**15**	0,038	0,710	0,366	0,106	0,044	0,122	0,939	1,656	0,208
KF CRM 56	**16**	0,128	0,951	0,199	0,230	0,041	0,576	0,306	0,389	0,547
KF OTHER	**17**	0,746	0,760	0,584	0,695	0,244	0,146	0,165	1,149	2,637
KF OTHER	**18**	0,300	0,306	0,451	0,000	0,058	0,643	0,351	0,265	0,529
KF CRM 56	**19**	0,170	1,089	0,099	0,013	0,050	0,070	1,406	0,016	0,309
KF CRM 56	**20**	0,162	0,349	0,072	0,166	0,202	0,132	0,777	0,395	0,495
KF OTHER	**22**	0,029	0,231	0,335	0,147	0,068	0,429	0,185	0,535	0,045
NIR transmittance	**23**	0,064	0,076	0,123	0,118	0,025	0,502	0,061	0,146	0,101