



# **Routine Analytical Chemistry Sub-Group**

## **Technical Report**

### **2004 and 2006 Collaborative Studies for Ammonia in Tobacco**

**May 2008**

**Co-ordinators:**

Linda Crumpler (Chairman), R.J. Reynolds Tobacco Co., Winston-Salem, North Carolina

Linda Drake (Secretary and Study Co-ordinator), British American Tobacco, Southampton, UK

**Statistics:**

Linda Drake

## TABLE OF CONTENTS

1. BACKGROUND .....	3
2. SUMMARY.....	3
2.1 STUDY 1 (2004) .....	3
2.2 STUDY 2 (2006) .....	4
3. CONCLUSION.....	5
APPENDICES:.....	6
APPENDIX A .....	6
APPENDIX B .....	7
APPENDIX C .....	14
APPENDIX D .....	17
APPENDIX E .....	18
APPENDIX F.....	18
APPENDIX F.....	19
APPENDIX G .....	20
APPENDIX H .....	21
APPENDIX I.....	22
APPENDIX J.....	23

## 1. BACKGROUND

During 2002, the CORESTA Routine Analytical Chemistry (RAC) sub-group organised and carried out a collaborative study to determine ammonia in tobacco. This was part of a larger study that included nicotine, sugars, moisture and nitrate in tobacco. The main outcome of this study was that, in the absence of a CORESTA Recommended Method (CRM), there was not a common approach to the analysis of ammonia in tobacco amongst the sub-group labs.

During the September 2003 sub-group meeting it was agreed that the Industry had a need for a harmonised approach to the analysis of ammonia in tobacco. A suggestion was made that a questionnaire be drafted and sent to prospective participants requesting details on the various methods used to analyse for ammonia in tobacco. This was done in late 2003.

At the April 2004 sub-group meeting the different approaches highlighted in the questionnaires were presented alongside an outline proposal for a future harmonisation study protocol. A summary of the different approaches used is to be found in APPENDIX A. The group agreed with the proposal and RJR volunteered to draft a method and protocol with BATUK procuring and distributing suitable samples.

The method, protocol and 7 samples of ground tobacco were distributed to 16 volunteer sub-group laboratories during May/June 2004. Details of these can be found in APPENDICES B; C & D. **Laboratories were instructed to follow the protocol as closely as possible.**

The data from this 2004 study, along with the summary of the methods used, was presented at the September 2004 RAC sub-group meeting and is summarised in section 2.1.

However, the main outcome from this study was that there were insufficient laboratories following the protocol to provide data that could be used to calculate robust R & r statistics according to ISO 5725 in support of a new CRM for the analysis of ammonia in tobacco.

A decision was made at the October 2005 meeting to repeat the study but only involving laboratories who were able to strictly follow the protocol so that sufficient data could be obtained to support R & r statistical evaluation. The data from this 2006 study is discussed in section 2.2.

Historically, Continuous Flow Analysis (CFA) has been the preferred technique for this analysis, but more laboratories are now using the more sophisticated Ion Chromatography (IC). Laboratories were therefore invited during this second study to submit IC data if they were not able to analyse using CFA.

## 2. SUMMARY

### 2.1 STUDY 1 (2004)

The 7 samples had the following approximate concentrations:-

<b>Sample ID</b>	<b>% Ammonia (wwb)</b>
A	0.8
B	0.6
C	0.2
D	0.3
E	0.3
F	0.4
G	0.4

The proposed method is to be found in APPENDIX B with the protocol in APPENDIX C.

The list of participating laboratories is to be found in APPENDIX D.

16 sets of samples were distributed and 15 sets of results received within the required timescale. Thirteen of these had used the CFA technique, one a rapid method and one IC. Three of the laboratories had submitted obviously outlying data which were checked directly with the laboratories and because one of the laboratories (laboratory 6) admitted to having a technical problem, their data was removed from the study. The data from these 14 laboratories is to be found in APPENDIX E.

On examining the summary of the chemistries used by these laboratories (APPENDIX F) it was apparent the only 3 laboratories had strictly followed the protocol. This was clearly insufficient data to support the recommendation of a CRM for ammonia in tobacco.

During October 2005 a note was sent out to the sub-group asking how many laboratories would be in a position to strictly follow the protocol should there be a further study. 10 laboratories promised to follow the CFA protocol and 9 laboratories also indicated their willingness to test using IC. As these numbers were promising, the sub-group agreed to repeat the study, but only using 5 samples.

## **2.2 STUDY 2 (2006)**

In February 2006, 5 samples were duly despatched to the volunteer labs.

The 5 samples had the following approximate concentrations:-

<b>Sample ID</b>	<b>% Ammonia (wwb)</b>
A	0.8
B	0.6
C	0.3
D	0.3
E	0.4

The method and protocol was as per the first study, with instructions to the laboratories that had the capability to analyse using IC to use their own in-house method.

The list of participating laboratories for the second study is in APPENDIX G.

Of the 8 laboratories that used CFA, only 3 were able to strictly follow the protocol, which was disappointing as this meant that there would be insufficient data to support the recommendation of a CRM. The data from this second study is to be found in APPENDIX H. The table of the methods used is in APPENDIX I.

Of the 8 laboratories who had initially promised to use an IC approach, data was received from 3 labs. This data is to be found in APPENDIX H. The information on the IC methods used is in APPENDIX J. Even though one of the labs used a different analytical approach compared to the other two labs (mainly in terms of extraction and elution media), the overall results are very well in line with the CFA data. Moreover, the between-lab variability of the 3 labs using IC was very acceptable and no trend of the results was observed when comparing the two analytical approaches. The data consistency between the ammonia results obtained by CFA and IC has to be confirmed however on a larger scale with more participating IC labs.

### **3. CONCLUSION**

There is still insufficient data to support the recommendation of a CRM for Ammonia in tobacco despite two back to back studies carried out by the RAC sub-group. However, the group did feel that it is important to publish and make available the data and methods used from these studies, hence the issuing of this report.

**APPENDICES:**

**APPENDIX A**

**AMMONIA IN TOBACCO – SUMMARY OF THE RANGE OF APPROACHES  
USED DURING 2002 STUDY.**

<b>ASPECT</b>	<b>APPROACH</b>
Extraction concentration	1 g in 25 ml 0.1 g in 100 ml
Extraction medium	Aqueous Acidic
Extraction time	20 minutes 30 minutes 60 minutes
Filtration device	Whatman 40 paper 0.45 µm disc
Reference material	Ammonium sulphate Ammonium chloride
Standard range	0 to 12 ppm 0 to 70 ppm
Sample to wash ratio	1:0.7 1:3
Wavelength	640 nm 660 nm 670 nm

## APPENDIX B

### Tobacco – Method for the Determination of Ammonia – Continuous Flow Analysis Method

April 2004

#### 1. Scope

This method is intended for use in the quantitative determination of ammonia in aqueous extracts of tobacco matrices. This method is valid over a wide range of concentrations. The applicable range may be extended by changing the detector sensitivity, sample dilution, or amount of sample used.

#### 2. Principle

An aqueous extract of tobacco is prepared and ammonia is determined by the reaction with salicylate and hypochlorite in a buffered alkaline solution. Sodium Nitroprusside is used as a catalyst. The resulting color is measured at 660 nm.

#### 3. Equipment/Apparatus

##### 3.1 Equipment

- 3.1.1 Rapid Flow Analyzer (Model 300) System, Astoria-Pacific International or equivalent, consisting of:
  - Autosampler
  - Micropump
  - Cartridge Base
  - Photometer
  - Analytical Cartridges for ammonia
- 3.1.2 Astoria-Pacific FASPAC Data Acquisition Software or equivalent.
- 3.1.3 Gyrotory Shaker – New Brunswick Scientific – model G-10 or equivalent.
- 3.1.4 Dispenser – Wheaton Omnispense or equivalent.
- 3.1.5 Mettler AT200 Analytical Balance or equivalent.

##### 3.2 Apparatus

- 3.2.1 Erlenmeyer Flasks - 125-mL, with stoppers or equivalent.
- 3.2.2 Filter Paper - Schleicher & Schuell 560 pleated or equivalent.
- 3.2.3 Vials - Polyethylene with caps (10-mL) or equivalent.
- 3.2.4 Sample Cups – 2-mL, Fisherbrand 02-544 or equivalent.

#### 4. **Reagents**

- 4.1 Sodium Hydroxide – 20% solution (Lab Chem # LC24090-2) or equivalent.
- 4.2 Sodium Potassium Tartrate (Fisher # S387-500) or equivalent.
- 4.3 Sodium Phosphate, dibasic (Fisher # S374-500) or equivalent.
- 4.4 Sodium Hydroxide pellets (Fisher # S318) or equivalent.
- 4.5 Sodium Chloride (Fisher # S271-1) or equivalent.
- 4.6 Sulfuric Acid (Fisher # A300-500) or equivalent.
- 4.7 Sodium Salicylate (Fisher # S395-500) or equivalent.
- 4.8 Sodium Hypochlorite (Clorox bleach Note: Any commercially available brand of liquid bleach containing 5.25% hypochlorite and no other additives is sufficient).
- 4.9 Ammonium Sulfate (Fisher # A938-500) or equivalent.
- 4.10 Brij-35 surfactant (Astoria-Pacific # 90-0710-04) or equivalent

#### 5. **Prepare Test Portion of Sample for Analysis**

- 5.1 Accurately weigh approximately 0.5000 ( $\pm$  .01) grams of “as received” tobacco material into a clean, dry 125-mL Erlenmeyer flask. Record the weight.

#### 6. **Extract and Filter**

- 6.1 Add exactly 100 mL of deionized water. Stopper the flask and shake for 30 minutes at 300 rpm on an orbital shaker. Filter extract through Schleicher and Schuell 560 pleated filter paper into a 10-mL polyethylene receiving vial. Place cap on vial until ready to analyze. Capped vials can be stored overnight under refrigeration prior to analysis if necessary.

#### 7. **Set-Up Instrument**

- 7.1 Set up the ammonia RFA-300 (1 channel) instrument, including the analytical cartridge, photometer, and sampler, as shown in the flow diagram (Figure 1).
- 7.2 Turn power on to all units of the system. Start pump and flush system with deionized water containing surfactant ( ~ 1% w/v Brij) for a minimum of 15 minutes until bubble pattern has stabilized. Connect reagent pump tubes (except salicylate/nitroprusside) to reagents according to flow diagram (Figure 1). Do not connect the salicylate/nitroprusside reagent until the other reagents have pumped through the analytical cartridge. This reagent will precipitate in the analytical cartridge under acidic conditions. Reverse this procedure when disconnecting the reagent lines from the pump. If precipitate forms immediately shut down the pump. Using a syringe, carefully flush the analytical cartridge with buffer until lines/coils are clear of obstruction. **Note:** Refer to Manufacturer’s Operating Instructions for comprehensive operating protocol.



**8. Prepare Reagents**

- 8.1 Sampler Wash Solution (1 L):
- |                 |      |
|-----------------|------|
| Deionized Water | 1 L  |
| 30% w/v Brij-35 | 1 mL |

Add 1 mL of Brij to 1 L of deionized water.

- 8.2 Stock Sodium Hydroxide Solution, 20% w/v (1 L):
- |                  |       |
|------------------|-------|
| Sodium Hydroxide | 200 g |
| Deionized Water  |       |

Dissolve 200 g of sodium hydroxide in 600 mL of deionized water contained in a 1-L beaker. Quantitatively transfer to a 1-L volumetric flask, let cool to room temperature, and dilute to volume with deionized water. This reagent solution is commercially available and ready to use.

- 8.3 Stock Sodium Potassium Tartrate Solution, 20% w/v (1 L):
- |                           |       |
|---------------------------|-------|
| Sodium Potassium Tartrate | 200 g |
| Deionized Water           |       |

Dissolve 200 g of sodium potassium tartrate in 800 mL of deionized water contained in a 1-L beaker. Quantitatively transfer to a 1-L volumetric flask and dilute to volume with deionized water.

- 8.4 Stock Buffer Solution (1 L):
- |                           |      |
|---------------------------|------|
| Sodium Phosphate, Dibasic | 71 g |
| Sodium Hydroxide          | 20 g |
| Deionized Water           |      |

Dissolve 71 g of sodium phosphate, dibasic in 800 mL of deionized water contained in a 1-L beaker. Add 20 g of sodium hydroxide and stir until dissolved. Quantitatively transfer to a 1-L volumetric flask and dilute to volume with deionized water.

- 8.5 Working Buffer Solution (1 L):
- |                                      |        |
|--------------------------------------|--------|
| Stock Buffer Solution                | 200 mL |
| Stock Sodium Potassium Tartrate Sol. | 250 mL |
| Stock Sodium Hydroxide Solution      | 250 mL |

Add 250 mL of stock sodium potassium tartrate solution to a 1-L beaker containing 200 mL of stock buffer solution and stir. While stirring, slowly add 250 mL of stock sodium hydroxide solution. Quantitatively transfer to a 1-L volumetric flask and allow to cool to room temperature. Dilute to volume with deionized water. Add several drops of Brij-35 surfactant to portion being used.

- 8.6 Sodium Chloride/Sulfuric Acid Solution (1 L):
- |                       |        |
|-----------------------|--------|
| Sodium Chloride       | 100 g  |
| Sulfuric Acid (conc.) | 7.5 mL |
| Deionized Water       |        |

Dissolve 100 g of sodium chloride in 600 mL of deionized water contained in a 1-L volumetric flask. Add 7.5 mL of concentrated sulfuric acid, mix and allow to cool to room temperature. Dilute to volume with deionized water. Add several drops of Brij-35 surfactant to portion being used.

- 8.7 Sodium Salicylate / Sodium Nitroprusside Solution (1 L):
- |                      |       |
|----------------------|-------|
| Sodium Salicylate    | 150 g |
| Sodium Nitroprusside | 0.3 g |
| Deionized Water      |       |

Dissolve 150 g of sodium salicylate and 0.3 g of sodium nitroprusside in 600 mL of deionized water contained in a 1-L beaker. Quantitatively transfer to a 1-L volumetric flask and dilute to volume with deionized water. Store in an amber container. Add 5 or 6 drops of Brij-35 surfactant to the portion being used for analysis.

- 8.8 Sodium Hypochlorite Solution (100 mL):
- |                              |      |
|------------------------------|------|
| CLOROX ( 5.25% hypochlorite) | 6 mL |
| Deionized Water              |      |

Add 6 mL of CLOROX to 50 mL of deionized water contained in a 100-mL volumetric flask. Dilute to volume with deionized water and mix. Add several drops of Brij-35 surfactant before use and mix. Prepare fresh daily.

## 9. Prepare Standards

- 9.1 Stock Standard Solution, 100 mg/L NH<sub>3</sub>, (1 L):
- |                  |           |
|------------------|-----------|
| Ammonium Sulfate | 0.3898 g* |
| Deionized Water  |           |

$$* 100 \text{ mg/L} \times 1 \text{ L} \times \frac{(\text{NH}_4)_2\text{SO}_4 (132.53 \text{ gfw})}{2 \times \text{NH}_3 (17 \text{ gfw})} \times \frac{1}{1000 \text{ mg/g}} = 0.3898 \text{ g}$$

where:  $(\text{NH}_4)_2\text{SO}_4 \equiv 2\text{NH}_3 + 2\text{H}^+$

For the stock solution, accurately weigh 0.3898 g of dry ammonium sulfate (99%) and quantitatively transfer into a 1-L volumetric flask. Dilute to volume with deionized water. This solution contains 100 mg/L of ammonia (NH<sub>3</sub>).

- 9.2 Working Standards

For the working standards, accurately pipette volumes (according to the table below) of the stock solution into 100-mL volumetric flasks. Dilute each working standard to volume with deionized water and mix well. These standard solutions are stable for at least three months under normal laboratory conditions.

Volume of NH <sub>3</sub> Stock Solution	Concentration of NH <sub>3</sub>	Instrument Calibrant Factor*
2.0 mL	2.0 mg/L	0.02
5.0 mL	5.0 mg/L	0.05
10.0 mL	10.0 mg/L	0.10
20.0 mL	20.0 mg/L	0.20
30.0 mL	30.0 mg/L	0.30
40.0 mL	40.0 mg/L	0.40

\*The factor (mg/L x 0.1L x 100/1000) is stored in the standards table in the FASPAC software to calculate weight percentage.

where: weight % = mg x 100 / sample wt. (gm) x 1000 mg/gm  
0.1 L = 100 mL sample extraction volume.

Record the weight, lot number, and date prepared for traceability of standards used with this method.

## 10. Analyze Standards and Filtrate

- 10.1 Load the prepared sample extracts on the sampler tray in the exact order of the sample table. A general completed sample table includes the following:

Sync cup	(high standard)
W	(deionized water; baseline)
Standards	(C1 through C6)
W	(deionized water; baseline)
LC	(known check solution)
Samples	(including appropriate check samples)
LC	(known check solution)
Wash	(extraction solution)
W	(extraction solution)

- 10.2 Begin analysis of samples by starting the sampler and the computer data acquisition channel.

- 10.3 Acceptance Criteria for Standards  
Instrument calibration is achieved by analyzing the working standards. A linear least square regression of peak height versus concentration (mg / 100 mL) is performed and updated automatically as each standard is completed. If the calibration statistics are not acceptable, determine the cause before repeating the analysis of samples. The calibration plot should be linear through all data points, a similar slope to previous runs, and a correlation coefficient above 0.998.

Results are expressed as a weight percentage, as follows:

$$\% = \frac{[\text{Sample Peak Height} - \text{Linear Regression Intercept}] \times \text{Dilution Factor}}{\text{Linear Regression Slope} \times \text{Sample Weight}}$$

- 10.4 Acceptance Criteria for Sample Data  
Examine peak heights to ensure that no sample peak height exceeds the response of the highest standard. If this condition exists, dilute the sample such that the response is within the range of standards. Analyze the diluted samples using the correct dilution factor. Review control sample data for acceptability.

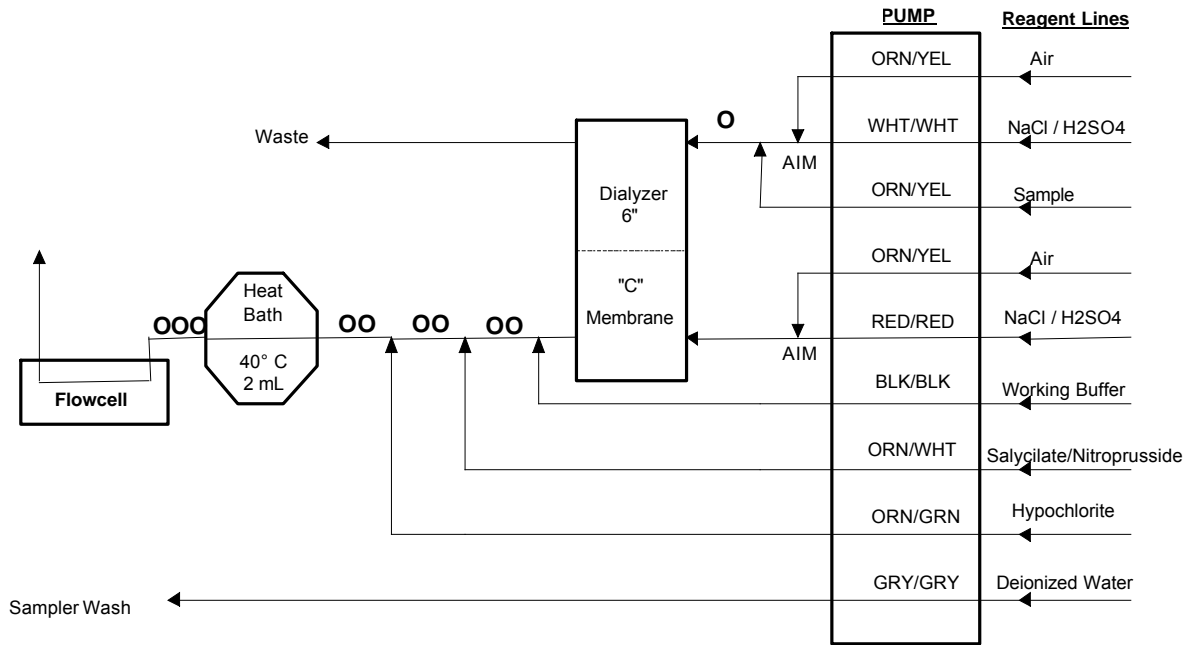
### Attachments:

Figures 1 and 2 - Ammonia Flow Diagram

Figure 1

**Flow Diagram for Ammonia in Tobacco**

Example of a continuous-flow analyzer,  $\mu\text{L}/\text{min}$  flow  
 Calibration Range: 0.2 mg/100mL - 4.0 mg/100mL



O = 1 Five Turn Coil  
 OO = 1 Ten Turn Coil  
 OOO = 1 Fifteen Turn Coil  
 AIM = Air Induction Module

(For variable speed pump, pump speed = 45)

**PHOTOMETER PARAMETERS**

Filter: 660nm  
 Flowcell: 10mm  
 Damping: 2.0

**SAMPLER PARAMETERS**

Analysis Rate: 90/hour  
 Sample Time: 20 sec.  
 Wash Time: 20 sec.

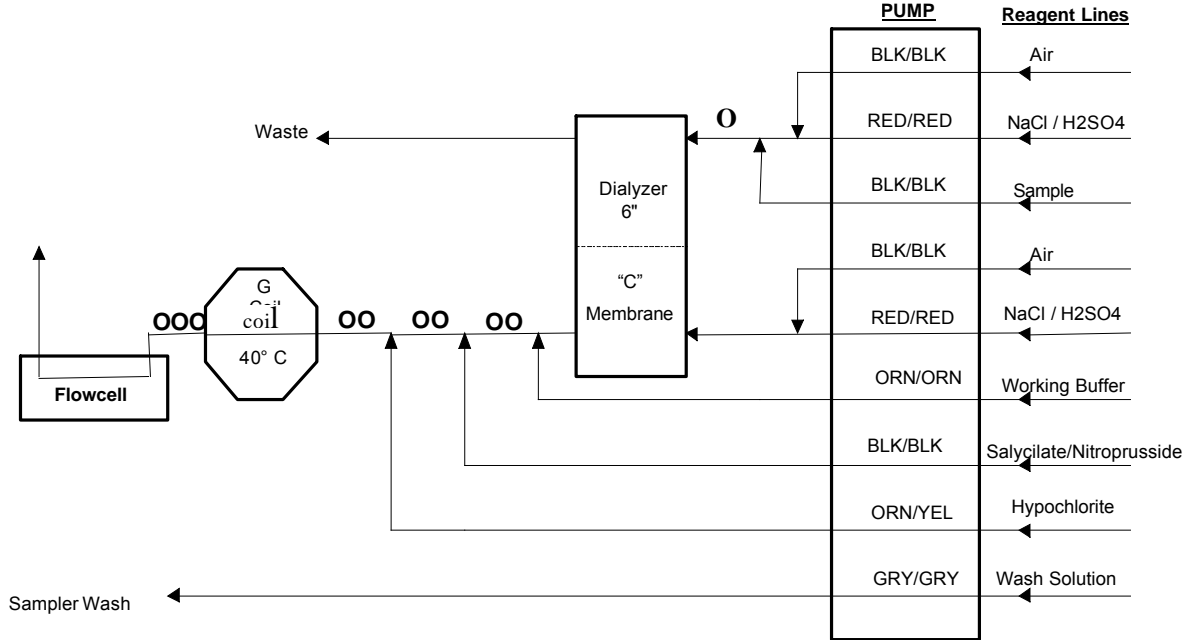
**PUMP TUBE FLOW RATES**

ORN/YEL = 118 $\mu\text{L}/\text{min}$ .  
 WHT/WHT = 385 $\mu\text{L}/\text{min}$ .  
 RED/RED = 482 $\mu\text{L}/\text{min}$ .  
 BLK/BLK = 226 $\mu\text{L}/\text{min}$ .  
 ORN/WHT = 166 $\mu\text{L}/\text{min}$ .  
 ORN/GRN = 74 $\mu\text{L}/\text{min}$ .  
 GRY/GRY = 568 $\mu\text{L}/\text{min}$ .

Figure 2

**Flow Diagram for Ammonia in Tobacco**

Example of a continuous-flow analyzer, mL/min flow



O = 1 Five Turn Coil  
 OO = 1 Ten Turn Coil  
 OOO = 1 Fifteen Turn Coil

Use YEL/YEL pump tube from Flow Cell to Waste

**COLORIMETER PARAMETERS**

Filter: 660nm  
 Flowcell: 10mm x  
 1.5mm ID

**SAMPLER PARAMETERS**

Analysis Rate: 40/hour  
 1:1

**PUMP TUBE FLOW RATES**

ORN/YEL = .16 ml/min  
 ORN/ORN = .42 ml/min  
 RED/RED = .80 ml/min  
 BLK/BLK = .32 ml/min  
 YEL/YEL = 1.20 ml/min  
 GRY/GRY = 1.0 ml/min

THIS DIAGRAM IS FOR INFORMATION ONLY.

## APPENDIX C

### PROTOCOL FOR INTERLABORATORY AMMONIA NITROGEN IN TOBACCO CROSSCHECK - 2004.

#### 1. INTRODUCTION

- 1.1 This procedure describes a method for conducting a study to determine the precision of the recommended Ammonia Nitrogen method as circulated in April 2004. Results are reported as % Ammonia, corrected to a dry weight basis using oven moisture.
- 1.2 The cross-check is designed as a **balanced uniform level experiment**, in which samples from 7 batches of materials, representing 7 different **levels** of the test are sent to participating laboratories. The laboratories have volunteered and are members of the CORESTA Routine Analytical Chemistry Sub-group.

The aim of the study is to assess the Repeatability and Reproducibility (see *Section 5 for definitions*) of all laboratories, and the Repeatability and Reproducibility of the Ammonia Nitrogen method with a view to recommending it for adoption by CORESTA as a Standard Method.

#### 2. SCOPE

- 2.1 This document describes how the cross-check procedure shall be conducted by an individual participating laboratory. This document does not describe the actual recommended test method for Ammonia Nitrogen, which is to be found in the method. **Laboratories will carry out the testing using method that reflects as close as possible the recommended method and note any deviations from the method when submitting their results.**
- 2.2 Statistical analysis of the data is not discussed in this document, but details can be obtained from Linda\_Drake@bat.com

#### 3. PROCEDURE

- 3.1 7 pouches of homogenised tobacco, at different ammonia nitrogen levels have been prepared by Southampton R&D, and distributed to the participating laboratories.
- 3.2 Each laboratory shall analyse **exactly 3** test portions, from each of the 7 pouches, **under repeatability conditions**:
  - i.e. within a short interval of time by the same operator, without any intermediate recalibration of the apparatus, unless this is an integral part of performing the measurement.

This will result in 3 measurements for each of the 7 pouches being reported in the spreadsheet (i.e. 21 measurements in all)
- 3.3 If an operator becomes unavailable, another one can complete the measurements, provided the change does not occur within a group of 3 tests but only between two of the 7 levels. Any such change shall be reported with the results.



## 5. DEFINITIONS

### 5.1 Repeatability:

The variability between independent test results obtained within a single laboratory in the shortest practical period of time by a single operator on the same set of test apparatus using test specimens from a single quantity of homogeneous material.

### 5.2 Reproducibility:

The variability between test results, obtained in different laboratories, using test specimens from a single quantity of homogeneous material.

### 5.3 Test result:

A test result is the value obtained by carrying out the complete test method once.



**APPENDIX D**

**STUDY 1 (2004)**

**LIST OF PARTICIPATING LABORATORIES**

Altadis France
Altadis S.A.
Arista US
BAT Germany
BAT UK
China National Tobacco
ETI (now BAT Italy)
KT&G
LTR Industries
PM International
PT HM Sampoerna
Reemstma/Imperial
RJRT
Rothmans B&H
Swedish Match

## APPENDIX E

## STUDY 1 (2004)

## RAW DATA

(Outlying laboratory 6 removed – technical problem)

Lab No.	Technique	SAMPLE A			SAMPLE B			SAMPLE C		
		1	2	3	1	2	3	1	2	3
1	CFA	0.750	0.750	0.760	0.550	0.560	0.560	0.180	0.180	0.180
2	CFA	0.798	0.788	0.799	0.612	0.628	0.624	0.213	0.207	0.207
3	CFA	0.850	0.840	0.840	0.610	0.610	0.610	0.190	0.190	0.190
4	CFA	0.833	0.825	0.824	0.585	0.584	0.579	0.190	0.189	0.189
5	CFA	0.810	0.812	0.839	0.590	0.638	0.595	0.200	0.201	0.202
7	CFA	0.917	0.924	0.896	0.632	0.635	0.629	0.200	0.202	0.200
8	CFA	0.840	0.832	0.833	0.617	0.604	0.606	0.190	0.190	0.186
9	CFA	0.810	0.820	0.820	0.590	0.590	0.590	0.180	0.190	0.190
10	CFA	0.800	0.810	0.790	0.560	0.580	0.560	0.200	0.210	0.190
11	CFA	0.893	0.883	0.871	0.602	0.602	0.590	0.186	0.186	0.196
12	CFA	0.855	0.847	0.854	0.589	0.596	0.571	0.190	0.191	0.191
13	CFA	0.834	0.841	0.878	0.584	0.608	0.623	0.202	0.202	0.207
14	RAPID ANALYSIS	0.798	0.832	0.806	0.590	0.605	0.571	0.178	0.179	0.183
15	IC	0.800	0.810	0.810	0.550	0.550	0.550	0.140	0.130	0.120
Lab No.	Technique	SAMPLE D			SAMPLE E			SAMPLE F		
		1	2	3	1	2	3	1	2	3
1	CFA	0.280	0.280	0.290	0.280	0.280	0.290	0.360	0.360	0.360
2	CFA	0.320	0.326	0.336	0.347	0.360	0.373	0.400	0.402	0.402
3	CFA	0.320	0.320	0.330	0.330	0.330	0.330	0.400	0.410	0.410
4	CFA	0.306	0.307	0.306	0.303	0.302	0.303	0.403	0.397	0.399
5	CFA	0.314	0.323	0.314	0.326	0.327	0.331	0.386	0.379	0.377
7	CFA	0.333	0.331	0.331	0.347	0.346	0.345	0.440	0.436	0.437
8	CFA	0.326	0.323	0.327	0.325	0.321	0.318	0.396	0.408	0.406
9	CFA	0.310	0.310	0.310	0.310	0.310	0.310	0.400	0.400	0.400
10	CFA	0.320	0.320	0.300	0.320	0.320	0.320	0.380	0.400	0.380
11	CFA	0.317	0.312	0.315	0.321	0.306	0.304	0.408	0.397	0.398
12	CFA	0.309	0.310	0.300	0.320	0.312	0.320	0.391	0.389	0.389
13	CFA	0.306	0.313	0.325	0.316	0.311	0.320	0.421	0.423	0.427
14	RAPID ANALYSIS	0.303	0.302	0.304	0.306	0.305	0.298	0.370	0.371	0.374
15	IC	0.260	0.260	0.260	0.260	0.280	0.280	0.370	0.360	0.360
Lab No.	Technique	SAMPLE G								
		1	2	3						
1	CFA	0.390	0.380	0.380						
2	CFA	0.381	0.397	0.379						
3	CFA	0.440	0.440	0.440						
4	CFA	0.402	0.406	0.405						
5	CFA	0.422	0.408	0.414						
7	CFA	0.438	0.437	0.437						
8	CFA	0.441	0.450	0.447						
9	CFA	0.390	0.390	0.390						
10	CFA	0.400	0.400	0.400						
11	CFA	0.455	0.459	0.466						
12	CFA	0.403	0.403	0.401						
13	CFA	0.434	0.430	0.444						
14	RAPID ANALYSIS	0.390	0.392	0.399						
15	IC	0.380	0.370	0.370						

**APPENDIX F****STUDY 1 (2004)****SUMMARY OF THE CHEMISTRIES USED BY THE 12 CFA LABORATORIES**

<b>LAB ID</b>	<b>METHOD FOLLOWED</b>	<b>COMMENTS/DEVIATIONS</b>
1	As protocol	1:100 dilution
2	CFA	Different reagents used.630nm.
3	CFA	5% Acetic acid extraction
4	As protocol	
5	Random Access Analyser	Reagents (DIC). 5% Acetic Acid extraction.
7	CFA	5% Acetic Acid extraction.
8	CFA	Reagents (DIC). Dil HCl extraction
9	CFA	1:100 dilution. Reagents (DIC). 2% Acetic Acid extraction.
10	As protocol	
11	CFA	Reagents (DIC).670 nm.
12	As protocol	
13	As protocol	Adjustment extraction volume

## APPENDIX G

### STUDY 2 (2006)

#### LIST OF PARTICIPATING LABORATORIES

Altadis France
Arista US
BAT Germany
BAT Italy
BAT UK
FTC
HVL
Labstat
Reemstma/Imperial
RJRT
Rothmans B&H

**APPENDIX H****STUDY 2 (2006)****RAW DATA**

Lab. No.	Technique	SAMPLE A			SAMPLE B			SAMPLE C		
		1	2	3	1	2	3	1	2	3
3	CFA	0.88	0.86	0.86	0.60	0.60	0.58	0.30	0.30	0.30
4	CRM	0.810	0.814	0.819	0.587	0.585	0.587	0.304	0.304	0.304
5	CFA	0.8842	0.8952	0.7703	0.6343	0.6020	0.6391	0.3507	0.3548	0.3716
6	CRM	0.810	0.830	0.840	0.600	0.570	0.590	0.280	0.300	0.300
8	CRM	0.806	0.830	0.836	0.589	0.593	0.597	0.304	0.302	0.302
10	CFA	0.82	0.82	0.82	0.58	0.59	0.60	0.30	0.32	0.31
11	CFA	0.750	0.720	0.750	0.580	0.490	0.470	0.240	0.230	0.260
13	CFA	0.790	0.820	0.830	0.600	0.570	0.600	0.310	0.310	0.310
14	IC	0.797	0.800	0.776	0.542	0.554	0.549	0.297	0.296	0.291
15	IC	0.840	0.900	0.900	0.680	0.670	0.660	0.300	0.280	0.280
16	IC	0.800	0.800	0.800	0.600	0.600	0.600	0.340	0.340	0.320
Lab. No.	Technique	SAMPLE D			SAMPLE E					
		1	2	3	1	2	3			
3	CFA	0.29	0.30	0.29	0.38	0.38	0.38			
4	CFA	0.297	0.301	0.295	0.388	0.383	0.387			
5	CFA	0.3375	0.3853	0.3734	0.4546	0.4317	0.4291			
6	CFA	0.300	0.290	0.290	0.380	0.380	0.390			
8	CFA	0.307	0.296	0.293	0.394	0.396	0.396			
10	CFA	0.30	0.30	0.30	0.39	0.38	0.40			
11	CFA	0.240	0.260	0.220	0.350	0.340	0.360			
13	CFA	0.290	0.290	0.290	0.410	0.410	0.410			
14	IC	0.268	0.273	0.268	0.391	0.384	0.391			
15	IC	0.390	0.380	0.380	0.350	0.330	0.350			
16	IC	0.330	0.330	0.330	0.420	0.420	0.420			

## APPENDIX I

## STUDY 2 (2006)

## SUMMARY OF THE CHEMISTRIES USED BY THE 8 CFA LABORATORIES

LAB ID	METHOD FOLLOWED	COMMENTS/DEVIATIONS
3	CFA	Extraction concentration and time. 5% acetic acid extract.
4	As protocol	
5	Random Access Analyser	Extraction concentration 5% acetic acid extract. Reagents. DCI.
6	As protocol	
8	As protocol	
10	As protocol	Modified Hypochlorite concentration
11	CFA	Buffer. Reagents. DCI.670 nm.
13	As protocol	Longer coil.

## APPENDIX J

### STUDY 2 (2006)

#### SUMMARY OF THE IC METHODS USED

		Lab 1	Lab 2	Lab 3
<b>Sample preparation</b>				
	<b>sample size</b>	ground sample (40 mesh)	Untreated	ground sample (<1mm)
	<b>sample weight</b>	100 mg	1000 mg	250 mg
	<b>extraction volume</b>	10 mL	40 mL	50mL
	<b>extraction solution</b>	0.025 N H <sub>2</sub> SO <sub>4</sub>	5 mM pyridine dicarboxylic acid + 20 mM tartaric acid (=mobile phase)	demineralised water
	<b>extraction time</b>	60 min	45 min	30 min @ 200 rpm
	<b>dilution</b>	1:5 or as appropriate	1:10 or as appropriate	1:10 or as appropriate
	<b>filtration</b>	0.45 µm	0.45 µm	0.45 µm
<b>IC analysis</b>				
	<b>column</b>	Dionex IonPac CS12A (4 x 250 mm x 8.5 µm)	Metrosep Cation C2 250 (4 x 250 mm x 7 µm)	Dionex IonPac CS12A (4 x 250 mm x 8.5 µm)
	<b>pre-column</b>	IonPac CG12A	Metrosep C2 250 guard	IonPac CG12A
	<b>elution</b>	tertiary gradient	isocratic	isocratic
	<b>eluent</b>	3 mM MSA (methanesulfonic acid) in water, water + H <sub>2</sub> SO <sub>4</sub> for cleaning	5 mM pyridine dicarboxylic acid + 20 mM tartaric acid	20 mM MSA (methanesulfonic acid) in water
	<b>detection</b>	autosuppression + conductivity detection	autosuppression + conductivity detection	autosuppression + conductivity detection
	<b>T° control</b>	no	no	no