Cooperation Centre for Scientific Research Relative to Tobacco

Special Analytes Sub-Group

CORESTA Recommended Method No. 83

DETERMINATION OF AMMONIA IN MAINSTREAM CIGARETTE SMOKE BY ION CHROMATOGRAPHY

May 2017

CORESTA RECOMMENDED METHOD Nº 83

Title:

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DETERMINATION OF AMMONIA IN MAINSTREAM CIGARETTE SMOKE BY ION CHROMATOGRAPHY

(May 2017)

0. INTRODUCTION

At the outset of this work, a survey conducted in the CORESTA Special Analytes Sub-Group determined that most laboratories used a method involving Ion Chromatography (IC) because they considered it most suitable so this was chosen as the basis of the recommended method. The method involved smoke collection using a combination of Cambridge Filter Pad (CFP) followed by impinger traps containing dilute sulphuric acid.

Other laboratories reported the application of an alternative trapping system consisting of impregnated and regular CFPs. The group decided to investigate both trapping systems.

Initial joint experiments and on-going discussions addressed some methodological aspects that needed to be considered before moving to a CORESTA Recommended Method (CRM). The draft CRM was produced in 2014 through a full Collaborative Study (CS) involving 17 laboratories from 8 countries using cigarettes manufactured from a range of blend styles (Virginia, American Blend and Dark Air-cured) that were smoked under both ISO and Health Canada Intense (HCI) smoking regimes. Both trapping systems were investigated and compared. Ammonia was determined by IC with conductivity or suppressed ion conductivity detection. Statistical evaluations were conducted according to ISO recommendations.

Results of the CS demonstrated equivalency of data obtained by using both trapping systems. From the results of CS, it was observed that the method was not fit for purpose for mainstream smoke generated from dark air-cured sample due to the high ammonia variation observed for both trapping systems.

The CRM includes both sample collection techniques, separation and detection conditions. It additionally provides recommendations to laboratories regarding features that need to be controlled to provide data as robust and consistent as the repeatability and reproducibility data indicated.

1. FIELD OF APPLICATION

This method is applicable to the determination of ammonia in mainstream cigarette smoke as the ammonium ion, using IC with conductivity or suppressed conductivity detection.

The method described is found to be not applicable for determination of ammonia in mainstream smoke generated from dark air-cured cigarettes.

2. NORMATIVE REFERENCES

ISO 3308:2012

Routine analytical cigarette-smoking machine – Definitions and standard conditions.

ISO 3402:1999

Tobacco and tobacco products – Atmosphere for conditioning and testing

ISO 4387:2000

Cigarettes – Determination of Total and Nicotine-free Dry Particulate Matter Using a Routine Analytical Smoking Machine

ISO 5725-1:1994

Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions.

ISO 5725-2:1994

Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability (r) and reproducibility (R) of a standard measurement method.

ISO 8243:2013

Cigarettes – Sampling.

3. METHOD SUMMARY

- Cigarettes are smoked on a standard smoking machine that has been fitted with a glass fibre filter (Cambridge Filter Pad, CFP) followed by impingers (trapping system 1) *or* by a combination of an acid impregnated and an untreated glass fibre filter (trapping system 2).
- The CFP is then extracted with the impinger solutions *or* in case of the application of two filter pads extracted with diluted mineral acid.
- The extract is filtered using a 0,45 µm syringe filter.
- The filtrate is analysed by cation exchange chromatography followed by conductivity or suppressed ion conductivity detection.

4. APPARATUS AND EQUIPMENT

Note: Polypropylene volumetric flasks, sample flasks, vials and storage containers should be used to minimise sodium originated from borosilicate glassware.

General laboratory apparatus and equipment is needed and in particular the following items:

- Equipment for conditioning of tobacco products.
- Equipment for marking the butt length.
- Equipment for smoking of tobacco products complying with ISO 3308:2012.
- Impingers for trapping mainstream smoke (e.g. 70 ml nominal volume).
- Erlenmeyer flasks of appropriate volumes with ground glass stoppers (or equivalent for samples extraction).
- Polypropylene tubing (e.g. Nalgene or equivalent) 1/4” ID × 3/8” OD.
- Analytical balance capable of measuring to four decimal places.
- Volumetric flasks 25 ml, 50 mL, 100 mL and 1 L.
- Mechanical pipettes with disposable plastic tips 10 µL -100 µL or equivalent.
- Dispenser capable of delivering volume of 15 mL.

- Tweezers and gloves for transferring pads.
- Wrist-action shaker or equivalent.
- Syringe filter – 0,45 µm nylon or equivalent.
- Disposable syringes – 5 mL or equivalent.
- Disposable glass Pasteur pipettes or equivalent.
- Rubber bulbs.
- Autosampler vials, caps and Teflon faced septa.

High Performance Liquid Chromatography (HPLC) system consisting of:

- A conductivity detector and conductivity suppressor (recommended - dependent on manufacturer).
- An eluent degassing unit or equivalent.
- Gradient pump.
- Autosampler with appropriate sampling loop and a cooling unit.
- Data Collection System.
- Column: Dionex IonPac CS16A cation exchange analytical column (250 mm × 3 mm) or equivalent.
- Disposable Guard Column: Dionex IonPac CG16A cation exchange guard column or equivalent.

5. REAGENTS AND SUPPLIES

All reagents shall be, at the least, recognized as analytical reagent grade.

- Ammonium sulphate ($(NH_4)_2SO_4$) > 99 % purity.
- Sulphuric acid (H_2SO_4) > 96 % purity (*for trapping system 1*).
- Hydrochloric acid (HCl) > 36,5 – 38 % purity (*for trapping system 2*).
- Methanesulphonic acid (MSA) > 99 % purity.
- Deionised water. Resistivity >18,0 MΩ.cm @ 25 °C.
- Ethanol.

Warning notice: The testing and evaluation of certain products using this test method may require the use of materials and or equipment that could potentially be hazardous and this document does not purport to address all the safety aspects associated with its use. Anyone using this test method has the responsibility to consult with the appropriate authorities and to establish health and safety practices in conjunction with any existing applicable regulatory requirements prior to its use.

6. PREPARATION OF LABORATORY EQUIPMENT

Polypropylene equipment should be cleaned and dried in such a manner to ensure that contamination does not occur.

Note: It is extremely important that all possible sources of contamination are removed from the work area.

7. PREPARATION OF SOLUTIONS

7.1 Sulphuric Acid, 0,010 M - Impinger Solution (Trapping System 1)

- Carefully add 1,0217 g H_2SO_4 (96 % w/w) to some of deionised water, in a 1 L volumetric flask.
- Mix and make up the solution to volume with deionised water.
- Store the solution in a storage bottle at ambient temperature.

7.2 Sulphuric Acid, 0,1 M - Solution C (Ion Chromatography Eluent)

- Carefully add 10,216 g H_2SO_4 (96 % w/w) to some of deionised water, in a 1 L volumetric flask.
- Mix and dilute to 1 L with deionised water.

7.3 MSA 0,003 M - Solution A (Ion Chromatography Eluent)

- Carefully add 0,288 g MSA to 900 mL of deionised water, in a 1 L volumetric flask.
- Mix and dilute to 1 L with deionised water.

7.4 Sulphuric Acid, 0,01M – Ammonium Standards Preparation

- Carefully add 1,022 g H_2SO_4 (96 % w/w) to min. 500 mL deionised water, in a 1 L volumetric flask.
- Mix and dilute to 1 L with deionised water.

7.5 Hydrochloric Acid, 0,05 M - Solution for CFP impregnation (Trapping System 2)

- Carefully add 4,3 mL HCl (36,5 % - 38 % w/w) to 500 mL ethanol in a 1 L volumetric flask.
- Mix and make up the solution to volume with deionised water.
- Store the solution in a storage bottle at ambient temperature.

7.6 Hydrochloric Acid, 0,01 M – Extraction solution (Trapping System 2)

- Carefully add 0,9 mL HCl (36,5 % - 38 % w/w) to some of deionised water, in a 1 L volumetric flask.
- Mix and dilute to 1 L with deionised water.
- Store the solution in a storage bottle at ambient temperature.

8. PREPARATION OF STANDARDS

8.1 Primary (1°) Ammonium Stock Solution

- Accurately weigh 0,10 g $(NH_4)_2SO_4$ into a 25 mL volumetric flask. Note the exact weight in order to accurately calculate the standard concentration.
- Dissolve in 0,01 M H_2SO_4, mix and make up to volume with 0,01 M H_2SO_4.

- This solution is stable for approximately 30 days when stored in refrigerator below 4 °C.

  Note: This corresponds approximately to a 1000 µg/mL ammonium ion stock solution.

8.2 Calibration Standards and Working Solutions

- Take appropriate volumes (0,02 mL to 0,20 mL) of the 1° ammonium stock solution and dilute to the prescribed volumes with 0,01 M H_2SO_4 to prepare calibration standards. An example of calibration standards preparation is summarised in Appendix 1.
- Transfer to autosampler vials and cap.

  Note: All working standards are made to volume in order to achieve a concentration of 25 mM H_2SO_4.

- The calibration should cover the concentration range of interest.

  Note: For Trapping System 2 (impregnated CFPs) the calibration range may require adjustment.

9. SAMPLING

Sampling is performed in accordance with ISO 8243:2013.

10. TOBACCO PRODUCT PREPARATION

Conditioning of the cigarettes is done in accordance with ISO 3402:1999.

11. SMOKING MACHINE PREPARATION

11.1 Smoking Machine Setup

The smoking parameters for which the method has been studied are set out in ISO 3308:2012 and in the Health Canada regulations 1999.

Table 1: Smoking parameters applicable for the method

Smoking regime	Puff volume (mL)	Puff frequency (seconds)	Puff duration (seconds)	Ventilation Blocking (%)
ISO 3308:2012	35	60	2	0
Health Canada Intense (HCI)	55	30	2	100

A routine analytical cigarette-smoking machine complying with ISO 3308:2012 is required with the following modifications as detailed below.

11.2 Trapping Systems

Two different trapping systems can be used. Trapping system 1 consists of a CFP combined with impinger traps. Trapping system 2 consists of an impregnated and an un-treated CFP.

11.2.1 Trapping System 1 – CFP and Impinger Traps

- Assemble the ammonia mainstream apparatus on the smoking machine by connecting two impingers, each containing 15 mL of 0,01 M H_2SO_4, between the CFP and the puffing mechanism using appropriate tubing (Figure 1).
- With the CFP and impingers in place ensure no leaks are detected.
- With the CFP and impingers in place, check and adjust the puff volume drawn by the smoking machine at the cigarette end as described in ISO 4387:2000.

Note: The same impingers can be used to adjust each port (e.g. linear smoking) before smoking begins. Laboratories are advised to assure that contamination of trapping systems from the environment is avoided.

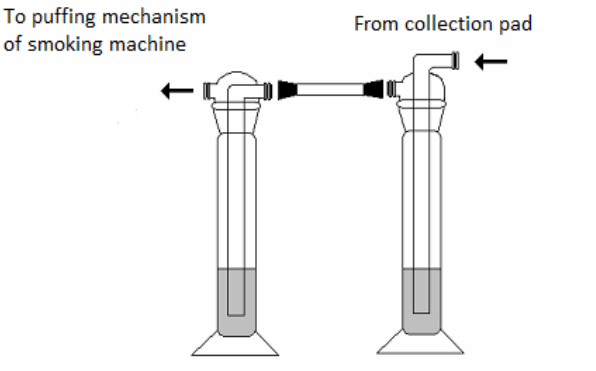

Figure 1: Example of Trapping System 1

Note: It is recommended to check the trapping efficiency when validating this method under both the ISO and Health Canada Intense smoking regimes to determine the suitability of the impingers that will be used. To check the trapping efficiency of the method, add an additional impinger and follow the method accordingly. Analyse each impinger individually for the compounds of interest. If no compounds are detected in the additional impinger then only the prescribed number of impingers is required to trap the vapour phase ammonia effectively, otherwise an additional impinger is required.

11.2.2 Trapping System 2 – Impregnated CFP

- Impregnate a CFP with 0,05 M HCl and place together with an untreated CFP in a filter holder connected to the smoking machine. The steps to prepare filter impregnation and trapping system are described below and further details can be obtained from Appendix 2.

11.2.2.1 Pre-treatment of CFPs

- Place a 44 mm standard CFP into a suitable container (e.g. culture vessel or a 100 mL beaker) containing 2,0 mL of ethanol/HCl aq. solution (concentration of HCl in mixture; 0.05M) and leave to soak.
- Place a 92 mm standard CFP into a suitable container (e.g. culture vessel or a 250 mL beaker) containing 6,0 mL ethanol/HCl aq. solution (concentration of HCl in mixture; 0.05M) and leave to soak.
- After being fully soaked, equilibrate impregnated CFPs for 2 h at 22 ± 1 °C and 60 ± 2 % of relative humidity.

Note: Refer to Appendix 2 for more details.

11.2.2.2 Assembly of Trapping System 2

- Place the impregnated CFP and the untreated CFP into the cigarette holder. The impregnated CFP should be in the front facing the incoming smoke and the untreated CFP at the back. The rough sides of both CFPs should face the incoming smoke.
- Assemble the cigarette holder in the smoking machine and connect the rear section of cigarette holder to the tubing leading to piston of smoking machine.
- Examine the system with a leak tester to ensure no air leaking is present.

Note: It is recommended to check the trapping efficiency when validating this method under both the ISO and Health Canada Intense smoking regimes to determine the suitability of the CFPs that will be used. To check the trapping efficiency of the method, add additional CFPs (treated and untreated) and follow the method accordingly. Analyse each CFP individually for the compounds of interest. If no compounds are detected in the additional CFPs then only the prescribed CFP trapping system is required to trap the vapour phase ammonia effectively, otherwise additional CFPs (treated and untreated) are required.

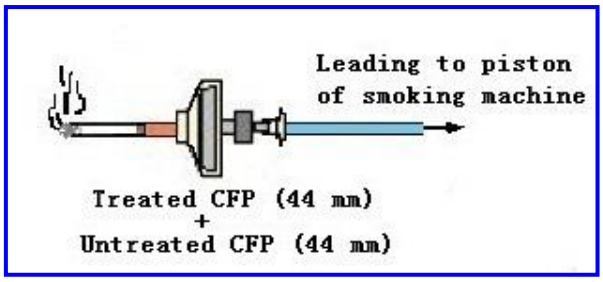

Figure 2: Example of Trapping System 2 (Linear Smoking Machine)

12. SAMPLE GENERATION – SMOKING OF CIGARETTES

The cigarettes are smoked according to ISO 4387:2000 with the following modifications:

- Check the puff volume of each port and adjust accordingly.
- Typically 5 cigarettes are smoked per replicate for ISO and 3 cigarettes per replicate for HCI smoking regimes using either a linear or a rotary smoking machine.

13. SAMPLE ANALYSIS

13.1 Extraction of CFPs - Trapping System 1

- After smoking remove the CFP, fold into quarters and place the pad into a 125 mL extraction flask.
- Add the solution from both impingers.
- Rinse the impingers with equal volumes of water (e.g. 30 mL impinger volume uses 30 mL of deionised water) and add to the extraction flask.
- Close the flask, and shake on the wrist action shaker for 30 minutes.
- Transfer the filtered extract to an autosampler vial.

- Samples can be stored in the refrigerator for up to 48 hours prior to analysis.

13.2 Extraction of CFPs - Trapping System 2

- After smoking, remove the two CFPs from the holder, fold into quarters and place into a 50 mL extraction flask containing 40 mL of diluted HCl solution (0,01 M).

Note. The volume of extraction solution was calculated for 44 mm CFPs. For 92mm CFPs the volume of extraction solution may require adjustment.

- Close the flask, and shake on the wrist action shaker for 40 minutes.
- Transfer the filtered extract to an autosampler vial.
- Samples can be stored in the refrigerator for up to 48 hours prior to analysis.

Note: If analysis of method blank is required, use "blank" CFP and impinger solutions for trapping System 1 and an impregnated CFP and un-treated CFP (not used for mainstream smoke trapping) for trapping system 2.

13.3 Sample Clean-up

- N/A

13.4 Cation Exchange Chromatography Analysis (Example)

- Examples of separation and detection conditions for analysis of samples obtained from both trapping systems are shown in the paragraphs below. The examples shown are applicable for Dionex equipment.

13.4.1 Chromatographic Conditions (Example)

- Column temperature: 30 °C.
- Autosampler equipped with cooling device: 4 °C ± 2 °C.
- Injection volume: 20 µL.
- Conductivity detector (Example – Dionex Suppressed Conductivity):

  Suppressor conductivity (SRS): 100 mA

  Scale: 10 µS

- **Mobile Phase / Gradient Conditions (Tertiary Gradient System)**

  Solvent A: 0,003 M MSA
  Solvent B: Deionised water
  Solvent C: 0,1 M H_2SO_4
  Flow: 1,5 mL/minute

Gradient:

Table 2: Example of a gradient

Time (minutes)	Composition		
	% A	% B	% C
0:00	100	0	0
13:00	100	0	0
13:01	0	80	20
14:00	0	80	20
14:01	0	90	10
19:00	0	90	10
19:01	0	99	1
20:00	0	99	1
25:00	99	1	0
25:00	Method End Action: Equilibrate (9 minutes)		

- Inject 20 µL of each sample onto the cation exchange column and analyse as per the chromatographic conditions listed above.
- Elution pattern should be similar to the example chromatograms shown in Figures 3 and 4.

Note: Adjustment to the operation conditions may be required if equipment from a different manufacturer such as Metrohm is used. However similar separation as shown on Figures 3 and 4 should be achieved.

13.5 Calculations

13.5.1 Calibration Curve:

- Each calibration standard (20 µL aliquot) is injected onto the column and analysed as per the relevant chromatographic conditions stated above.
- The calibration curve is fitted by a quadratic function. The response obtained for test samples should fall within the working range of the calibration curve.

13.5.2 Determination of Response Factor

- A calibration curve for ammonium is prepared by plotting the concentration of the standards versus the peak areas to determine appropriate response factors.

13.5.3 Sample Quantification

- The concentration of ammonia in smoke samples is quantified by the external standard method. The identification of peaks is by comparison of retention times with standards.
- Ammonium ion concentrations are reported in (µg/mL) by a chromatography software.

- Determination of mainstream smoke ammonia yields in µg/cigarette:

  Ammonium (µg/cigarette) = (Peak Area / Resp. Factor) × (DF / No. of Cigarettes)

  where:

  - DF is the dilution factor = Final Volume (e.g. (15 mL + 15 mL) × 2)
  - The Resp. Factor shall be determined from the calibration curve.

  Ammonia (µg/cigarette) = Ammonium (µg/cigarette) × 17/18

  where 17/18 corrects for the difference in molecular weight.

14. REPEATABILITY AND REPRODUCIBILITY

The Full Collaborative Study was conducted in 2014, involving 17 laboratories and five replicate analyses of 8 cigarette samples including the University of Kentucky reference cigarettes 3R4F and 1R5F and the CORESTA Monitor Test CM7 and covering a wide range of blends and cigarette design constructions.

Table 3: Overview of samples used in Collaborative Study

Sample	Product Characterisation	ISO Tar Yield (mg)
Sample 1	Dark air-cured product	9,5
Sample 2	American blended product	8,0
Sample 3	American blended product	6,4
Sample 6	Virginia blended product	9,7
Sample 7	Charcoal filtered / blended product	1,3
Ky 3R4F	Kentucky Reference 3R4F	8,2
Ky 1R5F	Kentucky Reference 1R5F	1,7
CM 7	CM7 Test Piece	14,2

Repeatability (r) and reproducibility (R) were calculated following the ISO 5725 Part 1 and 2 statistical procedures. R & r figures were calculated for ISO and Health Canada Intense smoking regimes and for the two trapping systems separately (Tables 4 – 7).

14.1 Ammonia ISO Smoking Regime Results

Table 4: Statistical data (µg/cigarette) for trapping system 1

Sample	MEAN	sr	sR	r	R	N
CM7	14,35	0,88	2,65	2,47	7,41	16
Ky 1R5F	2,55	0,27	0,71	0,77	1,98	15
Ky 3R4F	9,45	0,58	1,63	1,61	4,57	17
Sample 1	22,99	2,86	6,89	8,01	19,28	11
Sample 2	7,42	0,61	1,60	1,72	4,48	15
Sample 3	9,26	0,78	1,95	2,19	5,47	14
Sample 6	6,54	0,43	1,31	1,20	3,66	15
Sample 7	1,31	0,24	0,65	0,69	1,83	15

Table 5: Statistical data (µg/cigarette) for trapping system 2

Sample	MEAN	sr	sR	r	R	N
CM7	19,11	1,11	2,45	3,11	6,85	14
Ky 1R5F	2,77	0,34	0,71	0,95	1,99	14
Ky 3R4F	10,13	0,76	1,31	2,12	3,68	15
Sample 1	29,26	5,86	10,12	16,40	28,34	11
Sample 2	8,34	0,54	1,25	1,51	3,50	14
Sample 3	11,29	0,75	2,25	2,11	6,30	13
Sample 6	9,22	0,70	1,59	1,95	4,44	14
Sample 7	1,27	0,16	0,33	0,43	0,92	12

14.2 Ammonia Health Canada Intense (HCI) Smoking Regime Results

Table 6: Statistical data (µg/cigarette) for trapping system 1

Sample	MEAN	sr	sR	r	R	N
CM7	34,66	2,25	5,34	6,29	14,95	16
Ky 1R5F	28,45	1,87	5,20	5,24	14,55	16
Ky 3R4F	30,29	1,91	5,31	5,36	14,87	17
Sample 1	85,74	10,04	28,02	28,12	78,46	11
Sample 2	27,61	2,05	4,98	5,75	13,95	14
Sample 3	33,46	2,48	7,20	6,95	20,16	14
Sample 6	17,02	0,96	2,84	2,69	7,96	14
Sample 7	14,20	1,24	2,78	3,47	7,77	15

Table 7: Statistical data (µg/cigarette) for trapping system 2

Sample	MEAN	sr	sR	r	R	N
CM7	39,89	1,92	5,74	5,38	16,07	12
Ky 1R5F	30,38	2,75	4,57	7,70	12,79	15
Ky 3R4F	32,62	2,67	3,95	7,48	11,05	15
Sample 1	107,02	13,77	47,28	38,55	132,39	10
Sample 2	28,70	2,99	4,79	8,37	13,41	13
Sample 3	36,01	2,56	8,42	7,17	23,59	11
Sample 6	22,48	2,12	3,60	5,93	10,07	13
Sample 7	15,89	1,50	2,56	4,21	7,16	12

15. REPORT

15.1. Test results

- Ammonia yields in the mainstream smoke of cigarette in µg/cigarette were rounded to the nearest 0,01 µg.

16. BIBLIOGRAPHY

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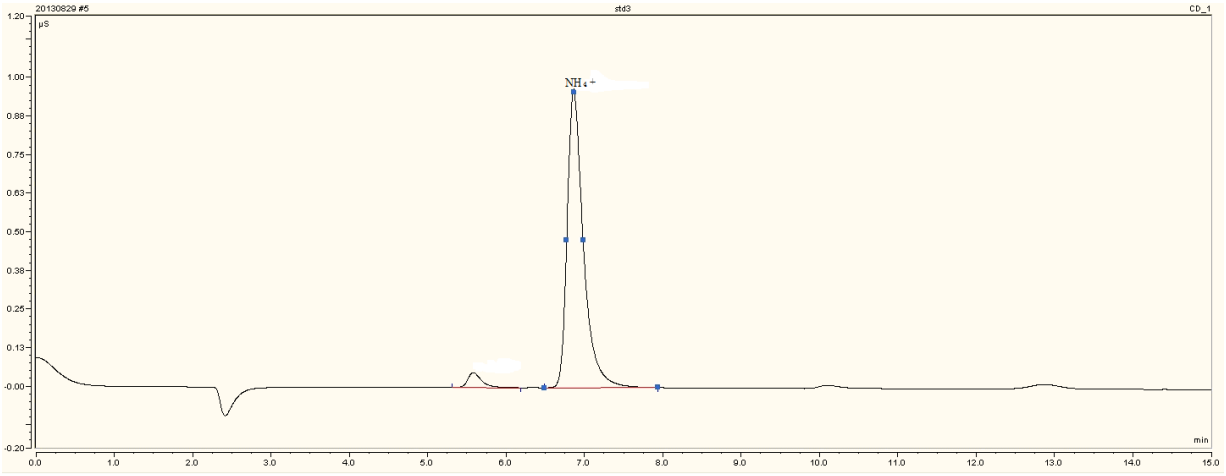

Figure 3. Example of Chromatogram of a Typical Calibration Standard using Dionex IonPac® CS16A Cation Exchange analytical column (250 mm × 3 mm)

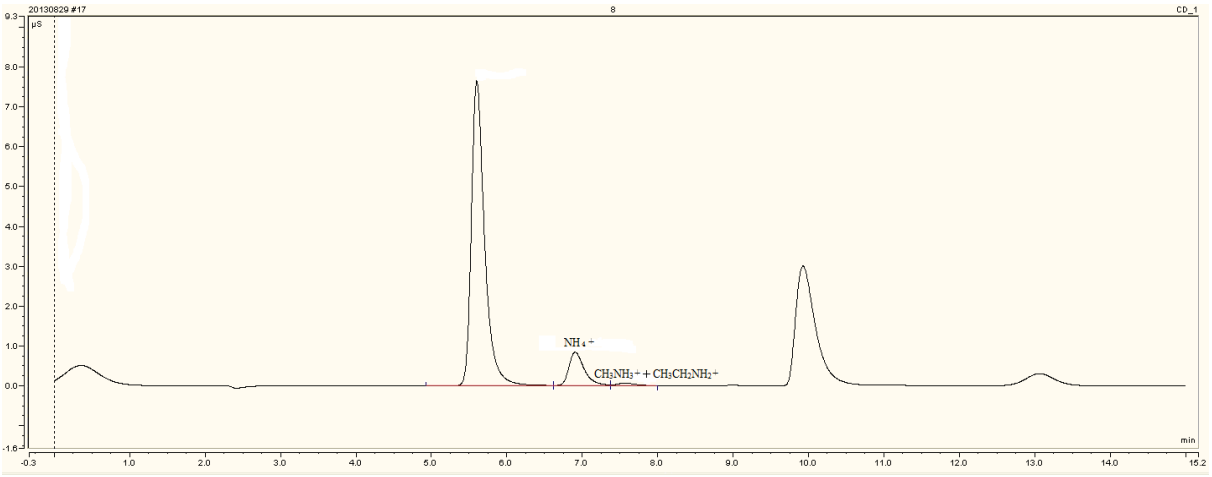

Figure 4. Example Chromatogram of Mainstream Cigarette Smoke Extract of Ky 3R4F (ISO Smoking) using the same Chromatographic Conditions as Figure 3

APPENDIX 1: Example of Preparation of Calibration Standards – Trapping System 1

- Assumption - Ammonium 1° Stock concentration is 1000 µg/mL

Standard #	Volume of 1° Standard (µL)	Final Volume (mL)	Concentration (µg/mL)
0	0	25	0
1	20	100	0,200
2	50	100	0,500
3	50	25	2,000
4	100	25	4,000
5	175	25	7,000
6	250	25	10,00

Note: All working standards should be freshly prepared every five days and stored in a refrigerator.

APPENDIX 2: Impregnation procedure of CFP (Trapping System 2)

A CFP (44 mm diameter for linear smoking machine and 92 mm diameter for rotary smoking machine) is placed in a suitable container (e.g. culture vessel or 100 mL beaker) and 1000 µl of ethanol/HCl aq. solution (concentration of HCl in mixture; 0.05M) is sprayed evenly drop by drop using a 100 µl - 1000 µl pipette. In total, 2000 µl and 6000 µL of ethanol/HCl aq. solution (concentration of HCl in mixture; 0.05M) solution is added to the 44 mm and 92 mm CFP, respectively.

Note: Alternatively, a novel small instrument named "Pad Sprayer" could be employed in the protocol, which facilitates this pre-treating procedure very quickly and glass fibre filter pads can be impregnated more evenly and quickly.

Once saturated, the impregnated CFPs are put into a container with holes on the bottom. The container is placed in a laboratory incubator and conditioned for 2 h at 22 °C ± 1 °C and 60 % ± 2 % of relative humidity.

Note: During the conditioning time it is necessary to keep the CFP at ISO conditions.The laboratory incubator in the protocol should be specifically used for the conditioning of the treated CFPs. Particularly, tobacco products should not be placed in the laboratory incubator to prevent CFPs from absorbing ammonia.

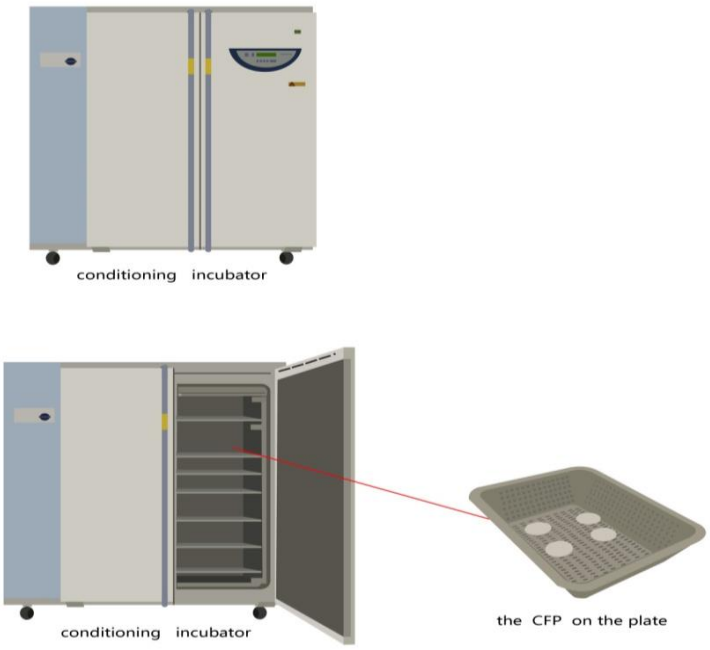

Figure 5. An example of conditioning cabinet and a container for CFPs conditioning

The effect of conditioning time of CFPs to the content of ammonia was assessed from 0,5h to 24h. As shown in the Figure 6, the amount of ammonia changed very slightly when the conditioning time increased from 0,5h to 8h both for 1R5F and 3R4F cigarettes. In case CFPs were conditioned for 12 hours the amount of ammonia was measured at a significantly lower level compared to results obtained for 8h conditioning time. Therefore it is recommended to condition the impregnated CFPs from 0,5h to 8h to ensure consistent conditions for measurement of ammonia in mainstream smoke.

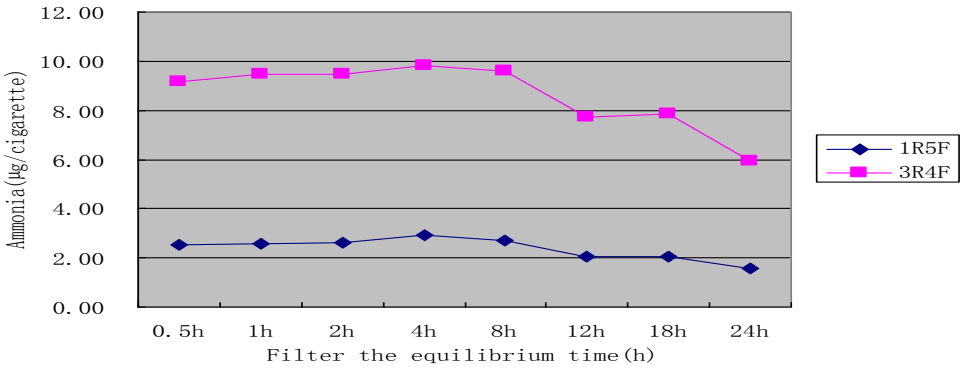

Figure 6. The content of ammonia for Ky 1R5F and Ky 3R4F cigarettes of different conditioning times