

CONFIDENTIAL

UK SMOKE CONSTITUENTS STUDY

Part 12: Determination of Polycyclic Aromatic Amines Yields in Cigarette Smoke

Annex A - method

Commissioned by:
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DEFINITIONS

The acronyms used in this procedure are listed and defined below.

| | |
|---------------|--|
| 1R4F | Industry Reference Cigarette Produced by the University of Kentucky |
| µg | Microgram |
| µL | Microliter |
| ALS | Automatic Liquid Sampler |
| amu | atomic mass unit |
| Cal | Calibration |
| Cigt. | Cigarette |
| Conc | Concentration |
| FTC | Federal Trade Commission |
| GC/MSD | Gas Chromatograph/Mass Selective Detector |
| ISO | International Organization for Standardization |
| ISTD | Internal Standard |
| LOD | Limit of Detection |
| LOQ | Limit of Quantitation |
| mL | Milliliter |
| MS | Mainstream Smoke |
| MSDS | Material Safety Data Sheet |
| N | Number of Replicates |
| ng | Nanogram |
| PAAs | 1-Naphthylamine, 2-Naphthylamine, 3-Aminobiphenyl and 4-Aminobiphenyl |
| PFFA | Pentafluoropropionic Acid Anhydride |
| PMP | Polymethylpentene |
| PTFE | Polytetrafluoroethylene (Teflon) |
| QC | Quality Control |
| RE | Relative Error |
| RSD | Relative Standard Deviation |
| SD | Standard Deviation |
| SIM | Selected Ion Monitoring |
| SOP | Standard Operating Procedure |
| Std | Standard |
| TMA | Trimethylamine |

THE DETERMINATION OF 1-NAPHTHYLAMINE, 2-NAPHTHYLAMINE, 3-AMINOBIIPHENYL AND 4-AMINOBIIPHENYL IN MAINSTREAM SMOKE

I. PURPOSE/SCOPE

This method describes the procedure for the determination of 1-Naphthylamine, 2-Naphthylamine, 3-Aminobiphenyl and 4-Aminobiphenyl (referred to, collectively, as "PAAs") in mainstream smoke.

II. PRINCIPLE OF METHOD

Mainstream (MS) smoke from test cigarettes is generated under conditions specified by the study protocol and passed through a 44-mm Cambridge filter. After smoking, the MS filter is weighed and transferred to a PMP Erlenmeyer flask containing 25 mL of 5% HCl. Internal standard is added and the sample is shaken for 30 min on a wrist action shaker. The extract is filtered into a separatory funnel and extracted with dichloromethane. The dichloromethane is disposed of as chlorinated waste and sodium hydroxide (50% solution in water) is added to the aqueous phase to make the sample basic. The contents are extracted three times with dichloromethane. The combined dichloromethane extract is derivatized with pentafluoropropionic acid anhydride (PFPA) and analyzed by GC/MSD after reacting overnight.

Individual analyte concentrations are determined by the internal standard method using gas-chromatography with Selected Ion Monitoring (SIM) detection. The concentrations of the analytes, determined by the GC/MSD are reported in units of mass-to-volume (i.e., ng/mL). The measured concentration, the number of cigarettes smoked, and the sample solution volume(s) are used to calculate the total analyte mass on a ng per cigarette basis.

The GC/MSD analysis uses a DB-5MS column for chromatographic separation of the analytes and selected ion monitoring (SIM) after electron impact ionization.

The analytical method used in this SOP has been fully validated and documented. The validation effort included trapping efficiency, selectivity, method accuracy and precision, detection and quantitation limits, and sample stability.

A flow diagram of the sample collection and workup procedure is given in Appendix A.

III. APPARATUS, CHEMICALS, AND LABORATORY SUPPLIES

A. Required Chemicals

See Appendix B:

B. Laboratory Apparatus and Supplies

- 44-mm Cambridge filter, part number 8 0 20 2851
- Automated liquid sampler (ALS) vials, with screw PTFE-lined lids.
- Volumetric flasks, calibrated "to contain" specified volumes, Class A.
- Volumetric glass pipettes, calibrated "to deliver" specified volumes, Class A.
- Laboratory balance with 0.1mg accuracy
- 125mL PMP Erlenmeyer flasks with screw-caps
- 125 mL glass Erlenmeyer flasks
- Wrist Action Shaker
- 100 – 1000 μ L Gas-tight Syringe(s)

- 250-mL separatory funnels
- Volumetric flasks – 10 mL, 25 mL, 50 mL, 100 mL
- Aluminum Foil
- Filter funnels, glass – 63 mm internal diameter
- Filter funnels, PMP – 95 mm internal diameter
- PH indicating paper
- Glass wool
- Mechanical dispenser, 1-100 μ L
- Pasteur Pipettes – nine inch (disposable) with rubber bulbs
- RapidVap, LABCONCO, cat# 79000-00
- 175 mL RapidVap vials
- Florisil cartridges, 12mL, 2000mg

IV. Preparation of Solutions

Precaution: PAAs are light sensitive. In the preparation of standards and samples, unnecessary prolonged exposure to light should be avoided.

A. Preparation of ISTD, Calibration and QC Solutions

See Appendices C, D & E.

V. Sample Collection and Workup

A. Sample Collection

Authors Comment – conditions for smoke generation and collection are described elsewhere – a summary is reproduced below.

- Cigarettes are conditioned¹ at a temperature of $22 \pm 1^\circ\text{C}$ and $60 \pm 3\%$ relative humidity for a minimum of 48 hours but not exceeding 10 days.
- Butt marking will be ISO butt length specifications². Filtered cigarettes will be smoked to a measured butt length equal to either the tipping paper + 3 mm or filter length + 8 mm whichever is longer. The minimum butt length will be 23 mm and this will also be used for non filter brands. All smoking shall be conducted in an environment of temperature $22 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ relative humidity¹.
- ISO conditions³ for smoking cigarettes will apply. The smoking machine puffing parameters will be $35 \pm 0.2\text{ cm}^3$ puff volume with 2.0 ± 0.05 second puff duration once every 60.0 ± 0.5 seconds.
- As a check that cigarettes have been smoked in accordance with ISO standard conditions, TPM yields were determined and compared with that normally achieved. Results for cigarettes that give significantly low or high TPM yields ($\pm 3 \times$ standard deviation) will be discarded.
- A minimum of five determinations will be performed for each brand. The smoking of the cigarette brands is randomised so that samples from the same brand are smoked on different days.
- With each batch of samples a 2R4F cigarette is smoked.

A flow diagram of the sample collection and workup procedure is given in Appendix A.

For mainstream smoke collection, the sampling apparatus will consist of a 44-mm Cambridge filter pad in a holder, connected directly behind the cigarettes to an analytical smoking machine.

Each mainstream sample will consist of the smoke collected from five cigarettes smoked under ISO conditions.

¹ ISO 3402: 2000 - Tobacco and tobacco products – atmosphere for conditioning and testing

² ISO :4387: 2000 - Methods for chemical analysis of tobacco and tobacco products – Determination of total and nicotine- free dry particulate matter using a routine analytical smoking machine

³ ISO 3308:2000 – Routine analytical cigarette smoking machine – 1: Definitions and standard conditions

VI. PROCEDURE

A. Extraction of Filter Pads:

1. Wrap 125 mL PMP Erlenmeyer flasks with aluminum foil to protect sample from light.
2. Remove the smoke pad from its holder, folding it into quarters and wiping the inside of the holder with the clean side of the pad.
3. Transfer the pad to a 125mL PMP Erlenmeyer flask containing 25 mL of 5% HCl.
4. Place screw cap tightly on the Erlenmeyer flasks.
5. Shake the sample on a wrist action shaker for 25 minutes.

B. Liquid/Liquid Extraction:

1. Thoroughly rinse the 95 mm ID PMP filter funnels and the separatory funnels with 5% HCl.
2. Wrap separatory funnels with aluminum foil to protect the samples from light.
3. Place the separatory funnels into the support stand.
4. Place glass wool plugs into the 95 mm PM{ filter funnels and place the filter funnels into the separatory funnels.
5. Add 100 μ L of internal standard to the extract in the 125 mL Erlenmeyer flask and shake thoroughly.
6. Pour the extract and as much of the remaining smoke pad as possible into the filter funnel and filter into the separatory funnel.
7. After the extract has finished filtering into the separatory funnel, rinse the Erlenmeyer flask twice with \sim 5 mL of 5% HCl and pour through the filter funnel into the separatory funnel.
8. Add 25 mL dichloromethane to the separatory funnel.
9. Stopper the separatory funnels and shake with careful venting and let settle.
10. Draw off the dichloromethane (bottom) layer and discard this layer as chlorinated waste.
11. Slowly add 10 mL 50% sodium hydroxide (NaOH) solution to the remaining phase in the separatory funnel. Mix gently with careful venting until venting no longer releases pressure.
12. Check the pH of the solution with pH indicating paper. If it is not basic, add an additional 2 mL of 50% NaOH and recheck the pH.
13. Place glass wool plugs in glass filter funnels and pour approximately 25 g of sodium sulfate into the funnels.
14. Wrap the 125 mL glass Erlenmeyer flasks with aluminum foil to protect the samples from light.
15. Add 20 mL dichloromethane to the remaining phase in the separatory funnels and shake very carefully with venting into the fume hood until there is no more release of pressure. Let settle.
16. Draw off the dichloromethane (bottom) layer of the contents in the separatory funnels through the sodium sulfate in the glass filter funnels and into the 125 mL Erlenmeyer flasks.
17. Repeat steps 15 and 16 twice (total of three extractions).
18. Rinse the sodium sulfate in the glass filter funnels with \sim 5 mL of dichloromethane, collecting the dichloromethane wash in the 125 mL Erlenmeyer flasks with the sample extracts.
19. Add 50 μ L of TMA solution (see Appendix F for instructions for preparing TMA solution) and 25 μ L of PFPA. Allow to react overnight.
20. The next day, rinse Florisil cartridges with \sim 5 mL of DCM and discard the rinses. Pour each of the samples through separate Florisil cartridges into 175 mL RapidVap vials.
21. Place the 175 mL RapidVap vials in the RapidVap, turn on the vacuum pump and operate the RapidVap at 25% speed, 35° C and 245 mBAR until the volume has been reduced to 1-1.5 mL.
22. Transfer the samples to ALS vials and analyze by GC/MSD.

VII. INSTRUMENT ANALYSIS

A. Sample Run Order

Sampling analysis order is as follows:

- 1) Calibration standards = Calibration Curve Standards (S1 to S6)
- 2) Check Std = Quality Control calibration check standard
- 3) Samples in sets of 15 or fewer (typically 10), check std between each set
- 4) Check Std at the end of the sequence

Analysis order will be designed so that an equal number of batched samples in groups of 15 or less be analyzed and bracketed by calibration check standards. For example, if there are 24 samples then 12 will be analyzed as the first batch of samples and 12 will be analyzed as the second batch.

B. GC/MSD Apparatus and Operation Parameters

The GC/MSD is calibrated using the standards of 1-naphthylamine, 2-naphthylamine and 4-aminobiphenyl. 3-Aminobiphenyl is not commercially available, and therefore, the concentration of 3-aminobiphenyl in the sample extracts is quantitated from a calibration curve generated from 4-aminobiphenyl assuming that the responses are equal. D₉-4-Aminobiphenyl is used as the internal standard for all analytes. The MSD is operated in the selected ion monitoring (SIM) mode for maximum sensitivity. 1-Naphthylamine and 2-naphthylamine are quantitated using the ion at a mass-charge-ratio (m/z) of 289 while m/z 142 is used as a confirming ion. 3-Aminobiphenyl and 4-aminobiphenyl are quantitated using m/z 315 while m/z 168 is used as a confirming ion. D₉-4-Aminobiphenyl is quantitated using m/z 324. No confirming ions are scanned for d₉-4-aminobiphenyl. The intensities of m/z 142, 168, 289, 315 and 324 are collected in a single range from 17 minutes to 25 minutes.

The conditions listed below may be modified by the analyst, with the approval of the lead chemist, in order to produce acceptable chromatography.

TABLE IX.1 GC/MSD Parameters

| Equipment / Parameter | Make | Model | |
|----------------------------|---|-------------------|--|
| Gas Chromatograph | Hewlett-Packard | 6890 Series | |
| Mass Selective Detector | Hewlett-Packard | 5973 Series | |
| Data Acquisition Hardware | HP Vectra | | |
| Operating System Software | Windows NT HP Chemstation | | |
| Analytical Column | J&W DB-5ms (30 m x 0.25 mm id x 0.25 µm film) | Part No. 122-5532 | |
| Injection Port Temperature | 250 °C | | |
| Injection Port Liner | 4-mm gooseneck | | |
| Injection Volume | 1 µL | | |
| Injection Mode | Splitless (purge time 0.5 min) | | |
| Helium Flow | 1.0 mL/min constant flow | | |
| Transfer Line temperature | 250 °C | | |
| MS Quadrupole temperature | 150 °C | | |
| MS Source temperature | 230 °C | | |
| Initial Oven temperature | 80 °C hold for 1.0 minutes | | |
| Temperature Gradient | 5 °C/minute to 200 °C 20 °C/ minute to 260 °C hold 5.0 minutes | | |
| Run Time | 33 minutes | | |

Table IX.2 Quantitation Ions (m/z) and retention times

| Analyte | Quantitation Ion (m/z) | ~ Retention Time (minutes) |
|---------------------------------|------------------------|----------------------------|
| 1-Naphthylamine | 289 | 17.65 |
| 2-Naphthylamine | 289 | 19.16 |
| 3-Aminobiphenyl | 315 | 22.77 |
| 4-Aminobiphenyl -d ₉ | 324 | 23.34 |
| 4-Aminobiphenyl | 315 | 23.41 |

Example chromatograms are depicted in Figures IX.1 through IX.2

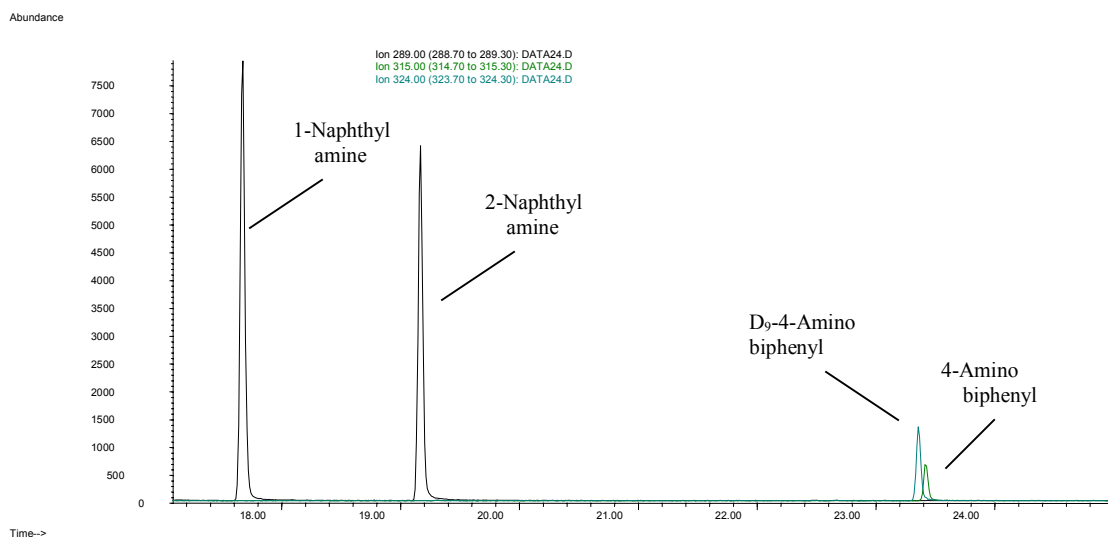


Figure IX.1 – Selected Ion Chromatogram for Ions 289, 315 & 324 for a S-4 Calibration Standard

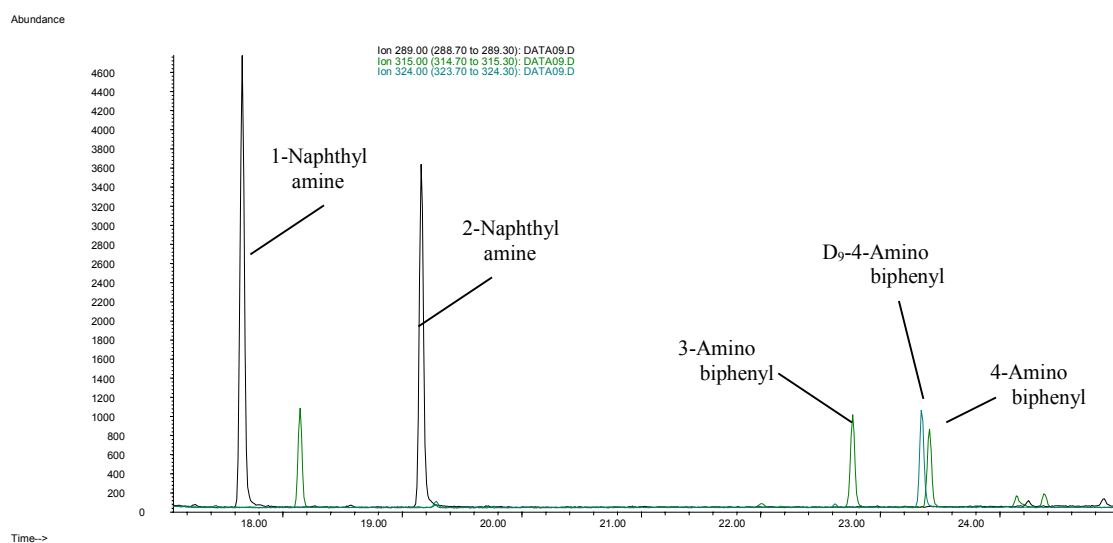


Figure IX.2 – Selected Ion Chromatogram for Ions 289, 315 & 324 for a 1R4F Smoke Sample

VIII. Data Reduction and Example Calculations

This section describes the calculations for the calibration of the GC/MSD and the method used for determining the amount of each analyte per cigarette.

A. Instrument Calibration Calculations

The analysis is carried out using the calibration capabilities of the HP Chemstation software and assumes the operator is already familiar with the procedure for setting up a calibration. Linear regression ($y = ax + b$) with equal weighting is used.

Both 3- and 4-aminobiphenyl are included in the calibration table. A calibration curve is built for both using the response and retention time of 4-aminobiphenyl in the calibration standards. The calibration curves should be identical. When the sample list is processed, the check standards will have results for 4-aminobiphenyl and the samples will have results for 3- and 4-aminobiphenyl.

B. Data Reduction of Raw Data from GC/MSD

The analyte concentration (in ng/mL) is determined by the internal standard calibration method using the regression equation derived from the calibration curve. The concentration of each analyte is obtained by using the calculation capabilities of the HP Chemstation software and assumes the operator is already familiar with the software operation.

The results generated by the Chemstation software are entered into the smoking data sheet for each sample. The operator must confirm that the correct values for sample dilution (typically 1 mL for MS) and number of cigarettes per sample (typically 5 or 3 for MS) are entered into the smoking data sheet. The number of cigarettes and the dilution volume are used to calculate the analyte delivery on a per cigarette basis.

Example Calculation:

$$\text{Amt analyte (ng/cigt)} = \frac{\text{Analyzed Conc(ng/mL)} \times \text{Volume(mL)}}{\text{Number of Cigarettes smoked(cigt)}}$$

A mainstream 1R4F sample had a 1-Naphthylamine concentration of 70.30 ng/mL. Assuming that 5 cigarettes were smoked and the final sample volume was 1 mL, the 1-Naphthylamine delivery for this sample is 14.06 ng/cigt.

$$1\text{-Naphthylamine (ng/cigt)} = \frac{70.30 \text{ ng.mL} \times 1 \text{ mL}}{5 \text{ cigt}} = 14.06 \text{ ng/cigt}$$

The percent recovery of all check standards is calculated as:

$$\% \text{ recovery} = \frac{\text{Analyzed Conc.} \times 100}{\text{Prepared Conc.}}$$

C. Data Acceptance

Each chromatogram is reviewed to confirm peak identification and integration using the Qedit software in the Chemstation package. If a peak must be manually integrated, the corrected value will be noted on the Chemstation sample summary sheet. A detailed report is generated for each sample.

In the event of poor chromatography (e.g., bad peak shape, no peaks, bad baseline, etc.), the lead chemist will be contacted. If the poor chromatography can be attributed to a single event, such as a bad injection, the standard or sample exhibiting the bad chromatography will be disregarded from any calculations, and a complete explanation will be included with the data.

D. Calibration and Quality Control Standard Acceptance Criteria

Each calibration curve must have a correlation coefficient (r^2) of 0.995 or better. Calibrations that do not meet these requirements should be brought to the attention of the lead chemist immediately. The analysis must stop, the problem must be corrected and the instrument re-calibrated. All calibration curves will be printed and copies stored with the corresponding data. The slopes of the curves will be compared to previous slopes and significant deviations (the amount of which has yet to be determined due to insufficient data) will be brought to the attention of the lead chemist.

All quality control standards must have a calculated amount between 90 and 110 % of the calculated values for each analyte. All quality control standards that do not meet these requirements should be brought to the attention of the lead chemist immediately. If a quality control standard is not $\pm 10\%$ of their calculated values the quality control standard may be replaced at the operators discretion with a different vial of the same standard and re-analyzed. If the new quality control standard is within $\pm 10\%$ of its calculated value then sample analysis may continue. If the new quality control standard is not $\pm 10\%$ of the calculated value then sample analysis must stop, the problem corrected and the instrument recalibrated. Any samples that are bracketed with a failed quality control standard must be re-analyzed.

E. Sample Reporting Criteria

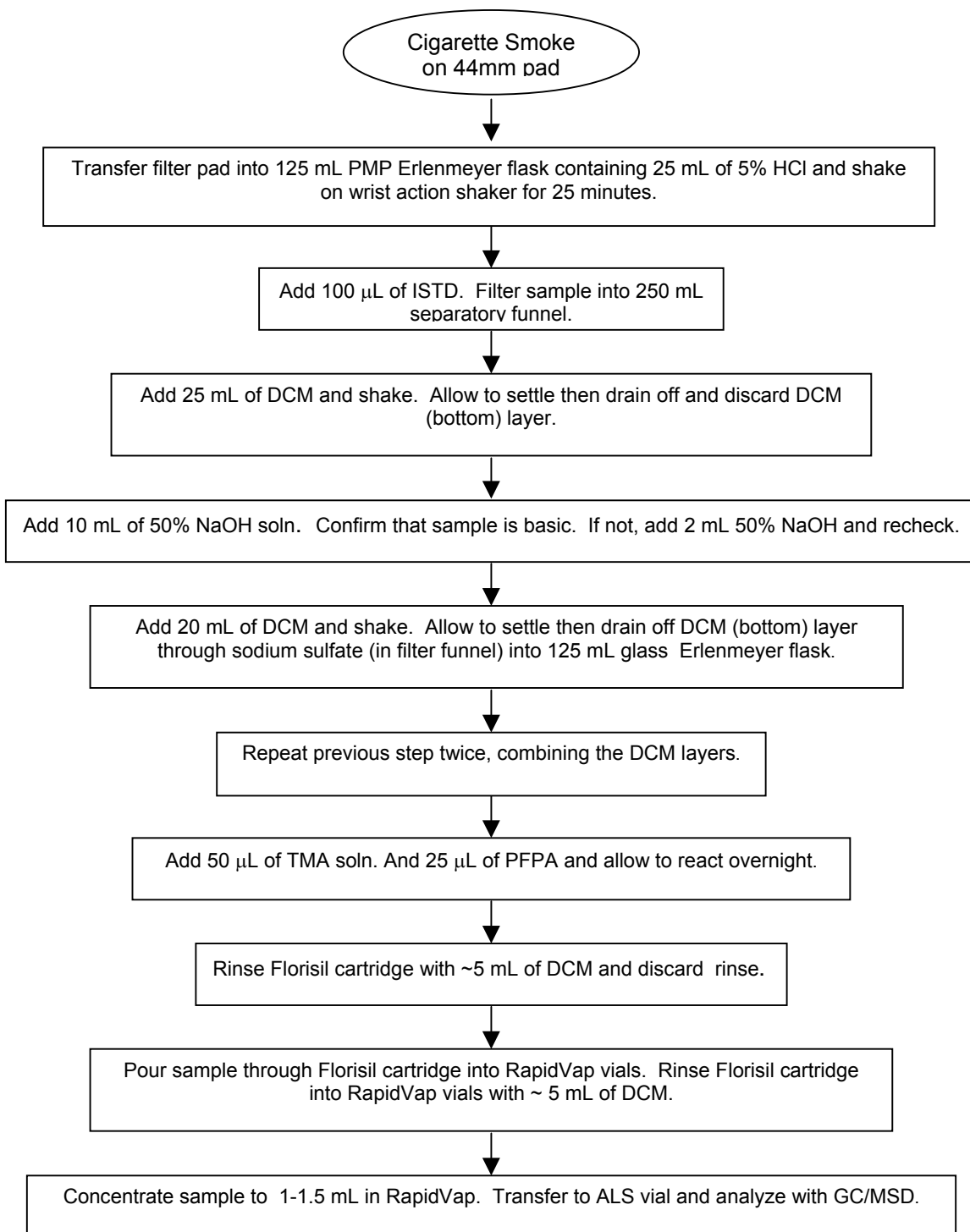
The method limit of quantitation (LOQ), and the method limit of detection (LOD) for the selected PAAs in mainstream samples were determined during method validation and are listed in Table X.1.

Table X.1: Method Detection and Quantitation Limits for Mainstream PAAs Analysis

| | | 1-Naphthylamine | 2-Naphthylamine | 4-Aminobiphenyl |
|---|--------------|-----------------|-----------------|-----------------|
| LOD and LOQ for Various Conditions: | | | | |
| | LOD(ng/mL) | 0.15 | 0.21 | 0.03 |
| | LOQ(ng/mL) | 0.50 | 0.70 | 0.10 |
| FTC/ISO | LOD(ng/cig)* | 0.03 | 0.04 | 0.01 |
| FTC/ISO | LOQ(ng/cig)* | 0.10 | 0.14 | 0.02 |
| *Note: Assumes a sample size of 5 cigarettes and 1mL final volume. | | | | |
| Mass/Mod ISO | LOD(ng/cig)* | 0.05 | 0.07 | 0.01 |
| Mass/Mod ISO | LOQ(ng/cig)* | 0.17 | 0.23 | 0.03 |
| *Note: Assumes a sample size of 3 cigarettes and 1mL final volume. | | | | |

Samples with concentrations outside of the calibrated range should be properly noted on the report data sheet. In the event the concentration of a sample is outside the upper limit of the calibrated range, a higher level of standard may be prepared. If there is insufficient sample to repeat the analysis, a repeat smoke sample may be taken with fewer cigarettes. See the lead chemist to determine the revised number of cigarettes to smoke.

APPENDIX A MAINSTREAM SAMPLE COLLECTION AND WORKUP PROCEDURE



APPENDIX B REQUIRED CHEMICALS

Use Table 1 for documentation of all required chemicals. Check labels carefully to ensure that all materials are current.

Table 1 – Required Chemicals

| Chemical | Supplier | Catalog Number | Certified Purity or Concentration |
|---|---|------------------|--------------------------------------|
| 1-Aminonaphthalene | Aldrich or equivalent | A6,639-1 | 95% |
| 2-Aminonaphthalene | Aldrich or equivalent | A6,640-5 | 95% |
| 4-Aminobiphenyl | Sigma or equivalent | A-2898 | 90% |
| 4-Aminodiphenyl-d₉ | C/D/N Isotopes or equivalent | D-2638 | 99% |
| Hydrochloric Acid (HCl) – 32% | Fisher or equivalent | A144s-212 | 32% |
| Dichloromethane (DCM) | Fisher or equivalent | D150-4 | HPLC grade |
| Sodium Hydroxide Solution – 50% | LabChem or equivalent | LC24140-4 | 50% |
| Pentafluoropropionic Anhydride (PFPA) | Aldrich or equivalent | 39,490-4 | 99% |
| Trimethylamine – 40% solution in water | Aldrich or equivalent | 43-326-8 | 40% |
| Sodium Sulfate – granular | Fisher or equivalent | S421-1 | |

APPENDIX C PREPARATION OF INTERNAL STANDARD SOLUTIONS

ISTD Solutions

Primary Stock (~1000 µg/mL):

Accurately weigh approximately 25 mg 1-aminobiphenyl-d₉ into a 25-mL volumetric flask. Record the actual amount to 0.0001 g. Bring to volume with dichloromethane. Mix well and transfer to an amber bottle with PTFE lined cap. This solution, stored at room temperature, expires after 6 months or whenever unacceptable standard curves or QC check standard results are obtained.

Secondary Stock (~4 µg/mL):

Add 0.1 mL of Primary Stock to a 25 mL volumetric flask. Bring to volume with dichloromethane. Mix well and transfer to an amber bottle with PTFE lined cap. This solution, stored at room temperature, expires after 6 months or whenever unacceptable standard curves or QC check standard results are obtained.

Tertiary Stock – MS ISTD Spiking Solution (~ 80 ng/mL):

Add 0.5 mL of Primary Stock to a 25 mL volumetric flask. Bring to volume with dichloromethane. Mix well and transfer to an amber bottle with PTFE lined cap. This solution, stored at room temperature, expires after 6 months or whenever unacceptable standard curves or QC check standard results are obtained.

Using a gas-tight syringe, or volumetric pipette, add 100 µL of the ISTD spiking solution to every 1 mL of final volume. For example, add 500 µL to each calibration standard and QC standard and 100 µL to each MS sample.

APPENDIX D: PREPARATION OF MS CALIBRATION STANDARDS

The storage conditions for the standard solutions are in amber bottles with Teflon-lined caps at room temperature. These solutions, stored at room temperature, expire after 6 months or whenever unacceptable standard curves or QC check standard results are obtained.

Primary (1°) Calibration Standard Stock Solution

Accurately weigh the approximate amounts listed in Table 2 into three 25-mL volumetric flasks (Class A). Record the actual amounts to 0.0001 g. Fill to the mark with dichloromethane and mix well.

Secondary (2°) Calibration Standard Stock Solution

Using a Gas-tight syringe, transfer the amounts of Primary Stock solution listed in Table 2 into a 25-mL volumetric flask (Class A). Fill to the mark with dichloromethane and mix well.

Tertiary Stock Solution

Using a Gas-tight syringe, transfer 500 uL of Secondary Stock solution into a 25-mL volumetric flask (Class A). Fill to the mark with dichloromethane and mix well.

MS Calibration Standards

Using a Gas-tight syringe, transfer 500 uL of the MS ISTD solution into each of six graduated test tubes. Add amounts of 3° Stock solution listed in Table 2. Add 10 µL each of TMA solution and PFPA. Add dichloromethane to bring total volume to 5 mL. Transfer to amber bottles with Teflon-lined caps and allow to react overnight. The next day, rinse Florisil cartridges with ~ 5 mL of DCM and discard the rinses. Pour each of the standards through separate Florisil cartridges into graduated test tubes. Rinse the Florisil cartridges with enough dichloromethane to bring the final volume to 5 mL. Transfer to ALS vials and cap with Teflon lined caps.

Table 1 – Excel Spreadsheet for recording MS Calibration Standards

Aromatic Amines Standard Solutions

1° Stock Solution

in methylene chloride

| | Target Wt. | Wt (mg) | Purity | 1° Stock Volume ml | 1° Stock Conc ug/ml |
|--------------------|------------|---------|--------|--------------------------|---------------------------|
| 1-aminonaphthalene | 100.0 | 90.8 | 0.95 | 25 | 3450 |
| 2-aminonaphthalene | 100.0 | 91.6 | 0.95 | 25 | 3481 |
| 4-aminobiphenyl | 25.0 | 46.1 | 0.90 | 25 | 1660 |

2° Stock Solution

- made to volume with methylene chloride

| | Vol 1° (mL) | 2° Stock Volume ml | 2° Stock Conc ug/ml |
|--------------------|-------------|--------------------------|---------------------------|
| 1-aminonaphthalene | 0.250 | 25 | 34.50 |
| 2-aminonaphthalene | 0.250 | 25 | 34.81 |
| 4-aminobiphenyl | 0.100 | 25 | 6.64 |

3° Stock Solution

- made to volume with methylene chloride

| | Vol 1° (mL) | 3° Stock Volume ml | 3° Stock Conc ng/ml |
|--------------------|-------------|--------------------------|---------------------------|
| 1-aminonaphthalene | 0.500 | 25 | 138.0 |
| 2-aminonaphthalene | 0.500 | 25 | 139.2 |
| 4-aminobiphenyl | 0.500 | 25 | 26.6 |

Working Standards (ng/mL)

| Label | Volume 3° Stock (mL) | Final Vol (mL) | Add 500 uL ISTD per 5.0 mL | | |
|-------|-------------------------|-------------------|----------------------------|----------------|----------------|
| | | | 1-amn ng/mL | 2-amn ng/mL | 4-abp ng/mL |
| S-1 | 0.05 | 5 | 6.90 | 6.96 | 1.33 |
| S-2 | 0.1 | 5 | 13.8 | 13.9 | 2.66 |
| S-3 | 0.25 | 5 | 34.5 | 34.8 | 6.64 |
| S-4 | 0.5 | 5 | 69.0 | 69.6 | 13.3 |
| S-5 | 1.0 | 5 | 138 | 139 | 26.6 |
| S-6 | 1.5 | 5 | 207 | 209 | 39.8 |

APPENDIX E : PREPARATION OF MS QC STANDARDS

The storage conditions for the QC standard solutions are in amber vials with Teflon-lined caps at room temperature. These solutions, stored at room temperature, expire after 6 months or whenever unacceptable standard curves or QC check standard results are obtained.

Primary Stock QC Solution

Weigh the approximate amounts listed in Table 3 into 25-mL volumetric flasks (Class A). Record the actual amounts to 0.0001 g. Fill to the mark with dichloromethane and mix well.

Secondary Stock QC Solution

Add the amounts listed in Table 3 of Primary Stock solution to a 25-mL volumetric flask (Class A). Fill to the mark with dichloromethane and mix well.

Tertiary Stock Solution

Using a Gas-tight syringe, transfer 500 uL of Secondary Stock solution into a 25-mL volumetric flask (Class A). Fill to the mark with dichloromethane and mix well.

MS QC Check Standard

Using a Gas-tight syringe, transfer solution 500 uL of the Secondary Stock QC Solution and 500 uL of the MS ISTD into a graduated test tube. Add 10 µL each of TMA solution and PFPA. Add dichloromethane to bring total volume to 5 mL. Transfer to an amber bottle with a Teflon-lined cap and allow to react overnight. The next day, rinse a Florisil cartridges with ~ 5 mL of DCM and discard the rinse. The QC standard solution through a Florisil cartridge into a graduated test tube. Rinse the Florisil cartridge with enough dichloromethane to bring the final volume to 5 mL. Transfer to ALS vials and cap with Teflon lined caps.

Table 2 – Excel Spreadsheet for recording MS QC Standards

Aromatic Amines Quality Control Std for 5 mL final volume

1° Stock Solution

in methylene chloride

| | Target Wt. | Wt (mg) | Purity | 1° Stock Volume ml | 1° Stock Conc ug/ml |
|--------------------|------------|---------|--------|--------------------------|---------------------------|
| 1-aminonaphthalene | 100.0 | 111.3 | 0.95 | 25 | 4229 |
| 2-aminonaphthalene | 100.0 | 95.6 | 0.95 | 25 | 3633 |
| 4-aminobiphenyl | 25.0 | 25.7 | 0.90 | 25 | 925 |

2° Stock Solution

- made to volume with methylene chloride

| | Vol 1° (mL) | 2° Stock Volume ml | 2° Stock Conc ug/ml |
|--------------------|-------------|--------------------------|---------------------------|
| 1-aminonaphthalene | 0.250 | 25 | 42.29 |
| 2-aminonaphthalene | 0.250 | 25 | 36.33 |
| 4-aminobiphenyl | 0.100 | 25 | 3.70 |

3° Stock Solution

- made to volume with methylene chloride

| | Vol 2° (mL) | 3° Stock Volume ml | 3° Stock Conc ng/ml |
|--------------------|-------------|--------------------------|---------------------------|
| 1-aminonaphthalene | 0.500 | 25 | 845.9 |
| 2-aminonaphthalene | 0.500 | 25 | 726.6 |
| 4-aminobiphenyl | 0.500 | 25 | 74.0 |

| QC conc. In 5 mL (ng/mL) | Add 500 uL ISTD per 5.0 mL | | | | |
|--------------------------|----------------------------|-------------------|----------------|----------------|----------------|
| Label | Volume 3° Stock (mL) | Final Vol (mL) | 1-amn ng/mL | 2-amn ng/mL | 4-abp ng/mL |
| QC Std | 0.5 | 5 | 84.59 | 72.66 | 7.40 |

**APPENDIX F :
PREPARATION OF HCL AND TMA SOLUTIONS**

5% Hydrochloric Acid solution

Add 312 mL of concentrated HCl to 1 liter of Type 1 water, dilute to 2 liters with Type 1 water.

TMA Solution

Add 2 mL of 40% trimethylamine solution to a large test tube containing 2 mL of hexane. Vortex, let settle and transfer hexane layer to a bottle containing ~ 1 gram of sodium sulfate. Cap and store at room temperature.