CORESTA RECOMMENDED METHOD N° 54

DETERMINATION OF NICOTINE AND NICOTINE-FREE DRY PARTICULATE MATTER IN SIDESTREAM SMOKE USING A FISHTAIL CHIMNEY AND A ROUTINE ANALYTICAL/LINEAR SMOKING MACHINE

(May 2011)

0. INTRODUCTION

Cigarettes are manufactured to close tolerances using strict quality control procedures. However, all the constituents involved in the manufacture are derived from natural products and this results in a final product which is intrinsically variable. Further complexity arises as the cigarette is converted during smoking to cigarette smoke.

The quantitative measurement of nicotine, of particulate matter and of nicotine-free dry particulate matter (NFDPM, sometimes referred to as "tar") is therefore dependent on the arbitrary definition of the means used to generate and collect the smoke. In particular, the ambient conditions (e.g., temperature, humidity, air movement within the laboratory) under which the test pieces are conditioned and smoke is collected, play a critical role in the accuracy of the measurement.

Sidestream Smoke in this Recommended Method is understood as the smoke that is evolved from the cigarette during the smoking run other than from the mouth end.

> ***Note:*** *this has to be distinguished from Environmental Tobacco Smoke (ETS), which is a mixture of aged and diluted exhaled mainstream smoke and aged and diluted sidestream smoke, and for the assessment of which the present method does not apply.*

From the time that scientists have attempted to determine nicotine and total and dry particulate matter yields in sidestream smoke, many different methods have been adopted. However, experience has shown some procedures to be more reliable and more amenable to handling of large numbers of samples. With these factors in mind, during the 1999-2002 period, collaborative studies by a Task Force composed of CORESTA members have shown that improvements in repeatability and reproducibility result when some restrictions are placed upon the wide variety of methods and practices described in existing methods.

This Recommended Method, produced after much collaborative experimentation by many laboratories in many countries, reflects the results of the optimizations proposed and validated by the Task Force and provides one set of procedures which are the accepted reference procedures and for which repeatability and reproducibility of the determinations were assessed. Experience in the Task Force has shown how the strict adherence to the detailed set up and conditions of the method as well as the degree of proficiency of the operator affect the precision of the results.

Further, the selected method should be compatible with different modes of cigarette equilibration or puffing parameters for the smoking of the tested pieces. The standards defined by CORESTA for the determination of mainstream yields were however followed to the largest possible extent, although the machines used by the different laboratories were all of a linear type.

This method is a machine method and it allows cigarettes to be smoked using a strictly controlled set of parameters. Thus it enables the sidestream NFDPM and nicotine from cigarettes, when smoked by this procedure, to be compared and ranked. In the course of its

studies, the Task Force demonstrated the value of comparing the analytical processes and their stability by use of the CORESTA monitor test piece for determining NFDPM and nicotine yields.

Since the determinations of NFDPM and nicotine in sidestream smoke are by nature more complex and delicate than their counterparts performed on mainstream smoke, it is highly recommended to include a control test piece in the smoking plans, as is done in mainstream determinations. It is possible to use the CORESTA monitor or any other internally designed control cigarette for this purpose. The use of an internationally recognized monitor test piece is recommended.

1. FIELD OF APPLICATION

This recommended method is applicable to the determination of total particulate matter and to the subsequent determination of nicotine and nicotine-free dry particulate matter present in the sidestream smoke from cigarettes. The described method is specified using the ISO 3308:2000 smoking parameters (puff volume, duration and frequency,) and butt length, but it is technically compatible with other smoking regimes. The method may not be directly applicable to other sidestream smoke analytes.

2. DEFINITIONS

For the purposes of this Recommended Method, the following definitions apply:

2.1. *Total Sidestream Particulate Matter: Crude Sidestream Smoke Condensate*

That portion of the sidestream smoke which is trapped on the sidestream pad, together with that portion of the sidestream smoke which condenses on the wall of the fishtail chimney, expressed as milligrams per cigarette.

2.2. *Dry Sidestream Particulate Matter: Dry Sidestream Smoke Condensate*

The dry sidestream particulate matter, expressed as milligrams per cigarette, and composed of the sum of:

the total particulate matter trapped on the sidestream pad after deduction of its water content,

plus the estimated nicotine-free dry particulate matter condensed on the walls of the sidestream chimney (this estimate being obtained by a UV absorption method to be described below),

plus the nicotine condensed on the walls of the sidestream chimney.

2.3. *Nicotine-Free Dry Sidestream Particulate Matter: Nicotine-Free Dry Sidestream Smoke Condensate*

The dry sidestream particulate matter, after deduction of its nicotine content, expressed as milligrams per cigarette.

2.4. *Sidestream Nicotine*

The sum of the nicotine condensed on the walls of the fishtail chimney, the nicotine collected on the sidestream pad and the nicotine collected in the impinger trap, expressed as milligrams per cigarette.

2.5. *Smoking Process*

The use of a smoking machine to smoke cigarettes from lighting to final puff.

2.6. *Smoking Run*

A specific smoking process to produce such sidestream smoke from a sample of cigarettes as is necessary for the determination of the smoke components.

2.7. *Laboratory Sample*

The sample intended for laboratory inspection or testing and which is representative of the gross sample or the sub-period sample.

2.8. *Conditioning Sample*

The cigarettes selected from the test sample for conditioning prior to tests for sidestream particulate matter and nicotine yield.

2.9. *Test Sample*

Cigarettes for test taken at random from the laboratory sample and which are representative of each of the increments making up the laboratory sample.

2.10. *Test Portion*

A group of cigarettes prepared for a single determination and which is a random sample from the test sample or conditioned sample as appropriate.

2.11. *Conditioned sample*

Conditioned cigarettes smoked for sidestream particulate matter and nicotine tests.

3. REFERENCES

ISO 2971:1998
Cigarettes and filter rods - Determination of nominal diameter

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

ISO 3400:1997
Determination of alkaloids in smoke condensates -- Spectrophotometric method

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 6488-1:1997, Tobacco -- Determination of water content -- Part 1: Karl Fischer method

ISO 6565:1999
Tobacco and tobacco products -- Draw resistance of cigarettes and pressure drop of rods -- Standard conditions and measurement

ISO 8243:1991
Cigarettes -- Sampling

ISO 10315:2000
Cigarettes – Determination of nicotine in smoke condensates – Gas-chromatographic method

ISO 10362-1:1999
Cigarettes – Determination of water in smoke condensates – Part. 1: Gas chromatographic method

ISO 13276:1997
Tobacco and tobacco products -- Determination of nicotine purity --Gravimetric method using tungstosilicic acid

CORESTA Recommended Method N° 7 (August 1991)
Determination of nicotine in the mainstream smoke of cigarettes by gas chromatographic analysis

CORESTA Recommended Method N° 8 (August 1991)
Determination of water in the mainstream smoke of cigarettes by gas chromatographic analysis

CORESTA Recommended Method N° 21 (August 1991)
Atmosphere for conditioning and testing tobacco and tobacco products

CORESTA Recommended Method N° 22 (August 1991)
Routine analytical cigarette-smoking machine specifications, definitions and standard conditions

CORESTA Recommended Method N° 23 (August 1991)
Determination of total and nicotine-free dry particulate matter using a routine analytical cigarette-smoking machine - Determination of total particulate matter and preparation for water and nicotine measurements

CORESTA Recommended Method N° 24 (August 1991)
Cigarettes – Sampling

4. PRINCIPLE

Sampling of the test cigarettes.

Conditioning of the test cigarettes.

Smoking of the test cigarettes on a smoking machine according to ISO 3308:2000, with the exception of the specifications on air velocity control, and equipped with a fishtail chimney, a glass-fibre filter pad and an impinger trap for each channel.

Simultaneous collection of total sidestream particulate matter on the walls of the fishtail chimney and in a glass-fibre filter pad, and collection of vapour phase sidestream nicotine in an impinger trap.

Gravimetric determination of the mass of total sidestream particulate matter collected on the glass-fibre filter pad.

Extraction of the total sidestream particulate matter from the glass-fibre filter pad for the determination of water and nicotine contents by gas chromatography.

Estimation of the nicotine-free dry sidestream particulate matter condensed on the walls of the fishtail chimney by a UV absorbance method.

Analytical determination by gas chromatography of the water collected in the glass-fibre filter pad.

Analytical determination by gas chromatography of the nicotine collected in the fishtail chimney, the glass-fibre filter pad and the impinger trap.

NOTE: In the countries that are not in a position to use the gas-chromatographic methods, reference should be made to ISO 3400:1997 for the determination of total alkaloids, and the determination of water in smoke condensate should be performed by the Karl Fischer method given in ISO 10362-2:1994. In such cases values obtained for nicotine and water in smoke condensate may be used with the addition of a note made in the expression of the result.

5. APPARATUS

Normal laboratory apparatus and in particular the following items:

5.1. *Fishtail chimneys*

Manufactured in glass, of design and dimensions shown in Figure 1 (Appendix).

NOTE: These fishtail chimneys are commercially available and may be obtained from, but not restricted to, Borgwaldt Technik GmbH and Cerulean.

5.2. *Routine analytical cigarette-smoking machine*

Modified to accept fishtail chimneys and complying with the requirements of ISO 3308:2000 with the exception of the specifications on air velocity control. A plate shall be fixed underneath each channel, with a minimum length of 120 mm and a minimum width of 50 mm. This plate is positioned so as to cover the totality of the opening at the fishtail chimney bottom, as shown in Figure 2 (Appendix).

5.3. *Impinger traps*

Dreschel gas-washing bottle, 125 ml, with open-ended stem, of the design and dimensions shown in Figure 2 (Appendix).

5.4. *A vacuum pump or pumps and flow control devices*

Capable of maintaining an air flow of 3 litres per minute through each fishtail chimney and collection train.

5.5. *PVC Tubing*

Tubing of approximately 8 mm I.D., 11 mm O.D., to connect the sidestream trap, impinger, in-line flow meter, flow regulator and vacuum pump.

5.6. *Flow monitoring and regulating system on each channel*

Comprising an in-line continuous-reading flow meter, capable of monitoring the flow with a resolution of 0.2 litre per minute, followed by a precision flow-regulating device.

5.7. *A primary flow meter*

Capable of accurately measuring a flow-rate of 3 litres per minute to a precision of 0.1 litre per minute, to be used in setting the air flow in each fishtail chimney before a smoke run. As this is a primary measurement, the flow meter should measure the time needed to flush a known volume.

5.8. *Soap bubble flow meter or alternative displacement flow meter*

Capable of measuring a displaced volume of at least the desired puff volume, with an accuracy of +/- 0.2 cm^3 and a resolution of 0.1 cm^3.

5.9. *Apparatus for the determination of puff duration and frequency*

5.10. *Analytical balance*

With a resolution of 0.1 mg.

5.11. *Draw resistance testing equipment*

As specified in ISO 6565:1999.

5.12. *Conditioning enclosure*

Carefully maintained in accordance with the conditions specified in ISO 3402:1999.

5.13. *Length-measuring device*

Suitable for measuring to the nearest 0.5 mm.

5.14. *Apparatus for the determination of diameter*

In accordance with ISO 2971:1998.

5.15. *Filter holder Sealing device*

End caps made from the same non-hygroscopic and chemically inert material as the filter holder.

5.16. *Gloves*

Cotton or non-talc surgical.

5.17. *Barometer*

Capable of measuring atmospheric pressures to the nearest 0.1 kPa.

5.18. *Laboratory shaker*

Capable of shaking at ca. 200 rpm

5.19. *UV spectrophotometer*

Preferably equipped with a batch sampler.

6. REAGENTS

Use only reagents of recognised analytical reagent grade.

6.1. *Propan-2-ol*

With maximum water content of 1.0 mg/ml.

6.2. *Internal standard for nicotine analysis*

n-heptadecane or quinaldine (of purity of at least 99%).

Carvone, n-octadecane or other appropriate internal standards may be used after assessment of their purity and determination that the internal standard does not co-elute with other components in the smoke extract, as described in ISO 10315:2000.

6.3. *Internal standard for water analysis*

Dried ethanol or methanol, (purity of at least 99%)

6.4. *Extraction solution*

Propan-2-ol (**7.1.**) containing an appropriate concentration of internal standards; this is normally in the range of approximately 0.2 mg/ml to 0.5 mg/ml for nicotine and 3.75 ml/l to 5 ml/l for water. Solvent not stored in a temperature-controlled laboratory shall be allowed to equilibrate to (22 +/- 2) °C before use. To prevent water being absorbed, the bulk solvent container shall be fitted with a water trap and all solutions shall be kept sealed. The solvent shall be stirred continuously to ensure the homogeneity of the water concentration in the solvent.

6.5. *Reference substance for nicotine*

Nicotine of known purity and verified in accordance to ISO 13276:1997. Store this at between 0°C and 4°C and exclude light. Nicotine salicylate of known purity and verified in accordance with ISO 13276:1997 may also be used.

6.6. *Reference substance for water*

Distilled or deionised water.

6.7. *Calibration solutions for nicotine and water in the sidestream glass-fibre filter pads*

Nicotine: Dissolve the nicotine (**6.5.**) in the extraction solution (**6.4.**) to prepare a series of at least four calibration standards for nicotine, whose concentrations cover the range expected to be found in the test portion (usually 0.2 mg/ml to 0.8 mg/ml).

Water: Prepare a series of least five calibration standards whose concentrations of added water cover the range expected to be found in the test portion (usually 0.2 mg/ml to 0.8 mg/ml) by adding weighed amounts of water (**6.6.**) to the extraction solution (**6.4.**). One of these calibration solutions shall be extraction solution with no added water (solvent blank). The calibration solutions shall be made up using an extraction solution from the same batch used for the test portion extraction. It is recommended that water calibration solutions be made up at least each week.

6.8. *Calibration solutions for chimney and impinger nicotine*

Prepare fresh with each batch of extraction solution (**6.4.**).

Prepare a series of at least five calibration standards for nicotine whose concentrations cover the range expected to be found in the chimney and impinger test portions (0.01 mg/ml to 0.2 mg/ml).

7. SAMPLING AND PREPARATION OF CIGARETTES

Provide a laboratory sample (see **2.7.**), by using a suitable sampling scheme. Guidance may be found in ISO 8243:1991. The sample will normally contain cigarettes taken from different parts of the population. Make up the test sample required for the test by randomly selecting cigarettes from the different parts of the population represented in the laboratory sample.

In **7.1.**, **7.2.** and **8.1.** below, the following symbols are used:

N is the number of cigarettes of a given type to be smoked, resulting from sampling at one point in time or from a sub-period sample.

C is a multiplying factor, value greater than 1, to allow for loss due to damage or selection procedures between initial sampling and smoking;

n is the number of replicate determinations of total sidestream particulate matter;

q is the number of cigarettes smoked into the same sidestream trap;

P is the total number of packets of cigarettes available;

Q is the total number of cigarettes available (laboratory sample, see **2.7**).

7.1. *Preparation of the Cigarettes for Smoking*

If N cigarettes of a given type are to be smoked, C x N cigarettes should be prepared from Q for conditioning and butt marking. The multiplier C is usually at least 1.2 to provide extra cigarettes in case some are damaged. If selection by mass or draw resistance (or any other parameter) is necessary, C will have to be much larger (experience suggests 2.0 to 4.0) depending on the selection process.

NOTE: The precision data given in this method are based on 8 replicates of 3 cigarettes. Any reduction in the number of replicates will affect the precision. It is not recommended to smoke less than 5 replicates.

The N cigarettes to be smoked will be tested in n = N/q determinations if q cigarettes are smoked into one trap. As far as possible these n determinations should correspond to different test portions of the test sample. Selection of each test portion will depend upon the form of the test sample.

7.2. *Selection of test portions of cigarettes*

7.2.1. *Selection of test portions from a bulk of Q cigarettes*

If the test sample is in the form of a single bulk, consisting of Q cigarettes, C x N cigarettes should be selected at random so that every cigarette has an equal probability of being chosen.

7.2.2. *Selection of test portions from P packets*

If the test sample consists of P packets, the selection procedure depends upon the number of cigarettes in each packet (Q/P) compared with q.

If $Q/P \geq C \times q$, select a test portion by choosing a single packet at random, then randomly select C x q cigarettes from that packet.

If $Q/P < C \times q$, select the smallest number of packets (k) such that

$$Q \times k / P \geq C \times q$$

and randomly choose an equal (or as near equal as possible) number of cigarettes from each packet to form the test portion of C x q cigarettes.

7.2.3. *Duplicate test portions*

Provided that the test sample is sufficiently large (≥ 2 C x N), it would be prudent to reserve a duplicate set of n test portions. In this event the parallel selection of a test portion and its duplicate would seem sensible. In this case the two selection conditions of Section 8.2.1 would need to be changed to

$$Q/P \geq 2 C \times q \quad \text{and} \quad Q/P < 2 C \times q.$$

7.3. *Marking the butt length*

7.3.1. *Standard butt length*

The standard butt length to which cigarettes shall be marked shall be the greatest of the following three lengths:

- 23 mm
- length of filter + 8 mm
- length of overwrap + 3 mm

where the overwrap is defined as any wrapper applied to the mouth end of the cigarette and the length of the filter is defined as the total length of the cigarette minus the length of the tobacco portion.

NOTE: Butt length is defined in ISO 3308:2000 as the length of unburnt cigarette remaining at the moment when smoking is stopped.

7.3.2. *Measurement of length of filter*

The length of filter as defined in **8.3.1.** shall be the mean value of 10 cigarettes taken from the laboratory sample measured to an accuracy of 0.5 mm. The mean shall be expressed to the nearest 0.5 mm.

NOTE: In some instances, it may be necessary to measure more than 10 cigarettes, but when the variation in filter length can be demonstrated to be well controlled, a smaller number of measurements may be sufficient.

7.3.3. *Measurement of length of overwrap*

The length of overwrap as defined in **8.3.1.** shall be the mean value of 10 overwraps taken from the laboratory sample measured to an accuracy of 0.5 mm. The mean shall be expressed to the nearest 0.5 mm.

NOTE: In some instances, it may be necessary to measure more than 10 cigarettes, but when the variation in overwrap length can be demonstrated to be well controlled, a smaller number of measurements may be sufficient.

7.3.4. *Butt length to be marked on the cigarettes before conditioning*

Draw a line, using a fine soft-tipped marker, at the standard butt length, to an accuracy of 0.5 mm, from the mouth end for the particular cigarette type.

Care should be taken to avoid damaging the cigarettes during butt marking. Any cigarettes accidentally torn or punctured during marking, or any found during marking to be defective, shall be discarded and replaced with spare cigarettes from the test portion.

If cigarettes are to be smoked on a smoking machine on which the butt length in accordance to **7.3.1.** can be pre-set, it is not necessary to mark the butt lengths on the cigarettes themselves.

7.4. *Selection of Cigarettes*

If a selection by mass or draw resistance (or any other parameter) is necessary because of the nature of the problem being studied, the selection is not to be considered as a method of reducing the number of cigarettes to be smoked.

7.5. *Conditioning*

Condition all the test portions in the conditioning atmosphere specified in ISO 3402:1999 for a minimum of 48 h and a maximum of 10 days.

If for any reason test samples are to be kept longer than 10 days store them in original packaging or in airtight containers just large enough to contain the sample.

The testing atmosphere in the laboratory where the smoking is to be carried out shall also be in accordance with ISO 3402:1999.

Transfer the test portions to the smoking location in airtight containers (just large enough to contain the portions) unless the smoking location and the conditioning location are adjoining and have identical atmospheres.

7.6. *Preliminary Tests before Smoking*

The following data may be required in the test report:

Total length of the cigarette.

Nominal diameter determined in accordance with ISO 2971:1998.

Draw resistance of the cigarette determined in accordance with ISO 6565:1999.

Average mass of the conditioned cigarettes selected for the smoking operation (milligrams per cigarette).

Water content (% mass/mass) of the conditioned cigarettes in accordance with ISO 6488-1:1997.

8. PREPARATIONS FOR THE SMOKING RUN

8.1. *Smoking Plan*

A smoking plan shall be chosen; examples are given in the annex to this Recommended Method.

The plan shall show the number of cigarettes to be smoked into each trap (q) and the number in the test sample for conditioning (C x N).

8.2. *Preparation of Mainstream and Sidestream Smoke Traps and Cigarette Holders*

For all operations the operator shall prevent contamination from the fingers by wearing gloves of a suitable material.

Prepare the mainstream smoke traps and cigarette holders according to ISO 3308:2000.

Insert into the sidestream smoke traps filter discs which have been conditioned in the test atmosphere for at least 12 h, and assemble placing the rough side of the filter disc so that it will face the oncoming smoke. After assembly, examine the filter holders to ensure that the discs have been properly fitted.

Weigh the assembled sidestream smoke traps to the nearest 0.l mg. Due to absorption of water by smoke traps and solvent, determine a value for the sample blank. Prepare sample blanks by treating additional smoke traps in the same manner as that used for smoke collection.

8.3. *Setting up the smoking machine*

Set up the smoking machine in accordance with ISO 4387:2000.

8.4. *Assembly of fishtail chimney, sidestream trap and impinger trap*

Each fishtail chimney shall be attached to an adjustable-height mounting block in such a way that it is securely held. Depending on the type of smoking machine and the degree of automation available, the mounting block may be manually or automatically raised and lowered. In its lowered position, the bottom of the fishtail chimney shall be a distance of 6 mm from the horizontal plate of the smoking machine. The raised position shall be at a height sufficient for convenient access for loading cigarettes and removing extinguished butts.

The sidestream glass-fibre pad holder is attached to the top of the fishtail chimney by means of a suitable connector or a short piece of vacuum tubing.

The sidestream impinger trap inlet is connected to the glass-fibre padholder by flexible tubing. This should be as short as practically possible and should be of polyvinyl chloride or, preferably, polyethylene polymer. Rubber or silicone rubber tubing shall not be used, as it

may absorb vapour phase nicotine. The sidestream impinger trap shall be held in a suitable clamp or clip. Conveniently, the impinger trap may be attached to the fishtail chimney mounting block. Its outlet is connected to the vacuum system by flexible tubing.

9. PROCEDURE FOR SMOKING RUN AND COLLECTION OF SIDESTREAM SMOKE

9.1. *Preparation of fishtail chimney*

Secure each fishtail chimney in its lower position, measuring the distance from the horizontal plate with a suitable 6 mm spacer. Raise the chimney to its upper position.

9.2. *Preparation of impinger trap*

Add 40 ml of 2-propanol containing the internal standard to each impinger trap, and connect the inlet to a sidestream glass-fibre pad holder by a short length of tubing. Connect each impinger trap outlet to the corresponding vacuum system.

9.3. *Setting the fishtail chimney flow rate*

Switch on the vacuum pumps. By means of the associated rotameters and needle valves, adjust the flow through each sidestream filter pad holder and associated impinger trap to 3.0 ± 0.1 litres/minute, using a suitable primary flow meter attached to the inlet of the sidestream filter pad holder. Switch off the vacuum pumps.

NOTE 1: If a soap bubble flow meter is used, the sidestream pad may absorb water during the flow adjustment procedure, and should therefore be reweighed before the smoke run begins.

NOTE 2: This procedure should be done as quickly as possible to minimise evaporation of the solvent from the impinger traps.

9.4. *Connection of sidestream glass-fibre pad holders*

Attach each sidestream trap securely to its fishtail chimney by means of a short piece of vacuum tubing or suitable connector.

9.5. *Record the atmospheric conditions*

Measure the temperature and relative humidity of the air surrounding the smoking machine and note the atmospheric pressure.

9.6. *Loading the cigarettes*

Insert the conditioned cigarettes into the cigarette holders to the insertion depth recommended in ISO 3308:2000 (9 mm). Avoid any leaks or deformations. Any cigarettes found to have obvious defects, or which have been damaged during insertion, shall be discarded and replaced with spare conditioned cigarettes.

Ensure that the cigarettes are positioned correctly so that the angle formed by the longitudinal axis of the cigarette and the horizontal plane shall be as small as possible. It shall not exceed 10° if the centre of the butt end is lower than the centre of the other end and 5° if the centre of the butt end is higher than the centre of the other end.

Adjust the position of each cigarette so that when the burning coal reaches the butt mark, the puff termination device (if applicable) is activated. If the burning through of 100 % cotton thread (40 denier) is used to terminate smoking at the butt mark, the cotton shall just touch the cigarettes at the butt mark, without modifying the cigarette positioning.

Ensure that the cigarette position is centred with respect to the fishtail, and that the fishtail covers a maximum length of the cigarette while ensuring that the distance between the end of

the cigarette and the front wall of the chimney is never less than 5 mm. In the case of long cigarettes this requirement may mean that the chimney may need to be moved along the axis of the cigarette as smoking progresses, in order to ensure that the fishtail covers the butt mark of the cigarette well before this is reached. The central axis of the cigarette will be positioned at a minimum of 15 mm above the bottom edge of the fishtail chimney

Return the fishtail chimneys to their lowest position compatible with the lighting system (a distance of about 60mm above the fixed plate has been found suitable).

9.7. *Smoking the cigarettes*

Switch on the vacuum pumps. Zero the puff counters and light each cigarette at the beginning of its first puff as specified by ISO 4387:2000. As each butt mark is reached, immediately raise the fishtail chimney and remove the burning coal from the cigarette. Record the final reading of the puff counters. After the smoking process is complete, allow the vacuum pump to run for a minimum of 30 seconds, in order to clear any sidestream smoke from the chimney.

If required, new cigarettes shall be inserted immediately and the smoking process repeated until the predetermined number of cigarettes (normally three) has been smoked on each channel. Begin the determination of total particulate matter as described in Section 11.

NOTE: Avoid disturbance of the smoking by artificial removal of ash. Allow ash to fall naturally into the ash tray.

10. DETERMINATION OF TOTAL PARTICULATE MATTER

10.1. *Total sidestream particulate matter retained on glass-fibre filter pads*

Wearing gloves, remove sidestream smoke traps from the smoking machine. Where necessary, remove the cigarette holder from the smoke trap.

Cover the front and back apertures of the traps with the sealing devices (5.15). Immediately after smoking weigh the smoke traps to the nearest 0.l mg.

Check the back of each filter disc to ensure that there are no brown stains indicating overloading or pad damage. Discard any disc showing such stains or damage. Forty four-mm glass-fibre filter pads are capable of retaining up to 150 mg of total particulate matter. If, during smoking, this mass is exceeded, the number of cigarettes shall be reduced and a calculation made to allow for the reduced number of cigarettes smoked. Since sidestream TPM yields of 30 mg are not unusual, smoking 3 cigarettes normally provides an adequate margin to prevent overloading the glass-fibre filter pads.

10.2. *Calculation of total sidestream particulate matter retained on glass-fibre filter pads*

The mean mass per cigarette of total sidestream particulate matter retained on the glass-fibre pad for each channel, T_p, expressed in milligrams per cigarette, is given by the equation:

$$T_p = (m_1 - m_0) \div q$$

where:

m_0 is the mass of the sidestream smoke trap before smoking (in milligrams)

m_1 is the mass of the sidestream smoke trap after smoking (in milligrams)

q is the number of cigarettes smoked into the trap.

NOTE: The total mainstream particulate matter may also be recorded, as it may serve as a check on the smoking procedure, although not directly the subject of this method.

10.3. *Treatment of total sidestream particulate matter retained on glass-fibre filter pads*

10.3.1. *Extraction procedure*

Wearing gloves, remove the sealing devices from the sidestream smoke trap, open it and remove the filter disc with forceps. Fold it twice, total particulate matter inwards, being careful to handle only the edge with forceps and gloved fingers. Place the folded disc in a dry conical flask (maximum flask volume 150 cm^3 for 44-mm discs). Wipe the inner surface of the filter holder front with two separate quarters of an unused conditioned filter disc and add these to the flask. Pipette 20 cm^3 extraction solution (propan-2-ol containing the internal standards for both nicotine and water determinations) into each flask.

Stopper the flask immediately and shake gently on a shaker for at least 20 minutes, ensuring that the disc does not disintegrate.

Follow the same procedure with each of the blank smoke traps used for the determination of water.

10.3.2. *Determination of Dry Sidestream Particulate Matter in the Glass-fibre filter pads*

Water in the supernatant solution in each flask is determined according to ISO 10362-1:1999.

The dry sidestream particulate matter retained in the glass-fibre filter pad, D_p, is calculated for each trap from the equation:

$$Dp = T_p - W_p$$

where:

D_p is the dry sidestream particulate matter, in milligrams per cigarette;

W_p is the water content in the total sidestream particulate matter, in milligrams per cigarette.

10.4. *Extraction of Sidestream Particulate Matter in the Fishtail Chimney*

The fishtail chimney is rinsed with extraction solution and the rinsings are collected in a 50-ml volumetric flask, which is then brought to volume, stoppered and reserved for UV absorption measurement and nicotine determination (see below).

NOTE: the rinsing should be done as soon as possible after completion of smoking. Where condensed matter cannot readily be solubilised, this may be aided by scraping carefully with a glass rod.

10.5. *Estimation of Sidestream Particulate Matter in the Fishtail Chimney*

NOTE: Chimney and sidestream pad extracts must be analysed within 36 hours of smoking.

10.5.1. *Principle of the method*

In order to obtain an estimate of the amount of sidestream nicotine-free dry particulate matter condensed on the fishtail chimney walls, the UV absorbance at 310 nm is measured for the fishtail chimney extract and the sidestream glass-fibre filter pad extract after appropriate dilution. At this wavelength, nicotine and water are not significant absorbers, and the ratio of absorbances, together with the initial volumes and dilution factors, enables the estimation of nicotine-free dry particulate matter condensed on the fishtail chimney walls. Appendix 2 gives informative examples of typical dilutions which may be applied for cigarettes of varying deliveries.

10.5.2. *Spectrophotometric analysis of the sidestream filter and fishtail chimney extracts*

Turn on the spectrophotometer at least 60 minutes prior to use. Set the wavelength to 310 nm. Zero the spectrophotometer with propan-2-ol.

Dilute the sidestream pad and chimney extracts as necessary. The absorbance measurement made for the determinations should not exceed 0.75 AU. Dilution of the sidestream filter extracts with propan-2-ol will normally be necessary, (except for the very lowest delivery cigarettes), to an extent dependent largely on the yields of the cigarettes (see appendix 3). The dilution factor applied to each extract should be carefully recorded. Obtain duplicate readings of the absorbance of each extract. Record readings for calculation.

The concentration of nicotine-free dry particulate matter (NFDPM) in the fishtail chimney extract is given by the equation:

$$x_c = (x_p \times a_c \times d_c) / (a_p \times d_p)$$

where:

x_c = concentration of NFDPM in chimney extract in mg/ml
x_p = concentration of NFDPM in sidestream pad extract in mg/ml
a_c = absorbance of diluted chimney NFDPM extract
a_p = absorbance of diluted sidestream pad extract
d_c = dilution factor applied to chimney NFDPM ' extract
d_p = dilution factor applied to sidestream pad extract

The amount of nicotine-free dry particulate matter in the chimney extract is then obtained by multiplying the concentration x_c by the volume of chimney extract (50 ml)

G_c is the estimated sidestream particulate matter condensed on the walls of the fishtail chimney, in milligrams per cigarette, and is calculated by the equation:

$$G_c = x_c \times 50 \div q$$

11. DETERMINATION OF SIDESTREAM NICOTINE

NOTE: It is recommended that capillary or megabore GC columns be used for the GC analyses listed below.

11.1. *Determination of nicotine in the sidestream glass-fibre filter pad (N_p)*

Nicotine in the supernatant solution in each flask obtained as described in section **10.3.1.** is determined according to ISO 10315:2000.

11.2. *Determination of Nicotine in the fishtail chimney extract (N_c)*

The solution obtained by washing the fishtail chimney (Section **10.4.** above) is analysed for its nicotine content as described in ISO 10315:2000.

11.3. *Determination of nicotine in the impinger trap (N_i)*

The content of the impinger trap is transferred to a 50 ml flask. The trap and the tubing connecting it to the glass-fibre filter are rinsed with a small volume of pure 2-propanol, and

the rinsings are added to the flask. The solution is then analysed for its nicotine content according to ISO 10315:2000.

NOTE: It is not necessary to make the solution up to a known final volume with pure 2-propanol, although this may be done if desired. The calculation of the amount of nicotine present may be made using the initial volume of 40 ml. It has been shown that although solvent is evaporated from the impinger trap during the smoking run, the internal standard is not lost to any significant extent.

11.4. *Calculation of Total Sidestream Nicotine (N)*

The total sidestream nicotine is calculated as the sum of the nicotine found in the fishtail chimney, the glass-fibre filter pad and the impinger trap, in mg per cigarette:

$$N = N_c + N_p + N_i$$

12. DETERMINATION OF NICOTINE-FREE DRY SIDESTREAM PARTICULATE MATTER

12.1. *Determination of nicotine-free dry sidestream particulate matter on the glass-fibre filter pad*

The nicotine-free dry sidestream particulate matter retained on the glass-fibre filter pad, G_p, is calculated for each glass-fibre filter pad from the equation:

$$G_p = D_p - N_p$$

where:

G_p is the nicotine-free dry sidestream particulate matter, in milligrams per cigarette which is trapped on the glass-fibre filter pad;

D_p is the dry sidestream particulate matter, in milligrams per cigarette;

12.2. *Determination of total nicotine-free dry sidestream particulate matter*

G is the total nicotine-free dry sidestream particulate matter, and is calculated from the equation:

$$G = G_p + G_c$$

13. TEST REPORT

The test report shall show the method used and the results obtained. It shall also mention any operating conditions not specified in this recommended method, or regarded as optional, as well as any circumstances that may have influenced the results.

The test report shall include all details required for complete identification of the sample. Where appropriate, record the information in **13.1. to 13.4.**

13.1. *Characteristic Data about the Cigarette*

Cigarette identification. In the case of a commercial cigarette this may include:

a) name of manufacturer, country of manufacture;

b) product name;

c) date of sampling;
d) place of purchase or sampling;
e) kind of sampling point;
f) sampling point (e.g. address of retail outlet or machine number);
g) packet number (of that product sampled that day);
h) marks on any tax stamp;
i) printed mainstream smoke yields (if any);
j) length of cigarette;
k) length of filter;
l) length of overwrap.

13.2. *Sampling*

Type of sampling procedure.

Number of cigarettes in laboratory sample.

Date and location of purchase.

13.3. *Description of Test*

Date of test.

Type of smoking machine used.

Type of smoke trap used.

Total number of cigarettes smoked in the entire determination on that cigarette type.

Number of cigarettes smoked into each smoke trap.

Butt length.

Room temperature (°C) during smoking operation.

Relative humidity (%) during smoking operation.

Atmospheric pressure (kPa) during smoking operation.

13.4. *Test Results*

The expression of the laboratory data depends on the purpose for which the data are required, and the level of laboratory precision. Confidence limits shall be calculated and expressed on the basis of the laboratory data before any rounding has taken place.

Average length of the cigarettes to the nearest 0.1 mm.

Average length of the filter to the nearest 0.1 mm.

Average length of the overwrap to the nearest 0.1 mm.

Butt length to which cigarettes were smoked to the nearest 0.1 mm.

Average lengths of tobacco portion smoked to the nearest 0.1 mm.

Average diameter of the cigarettes (mm).

Average draw resistance of the conditioned cigarettes.

Average mass (milligrams per cigarette) of the conditioned cigarettes selected for the smoking operation.

Water content (% mass/mass) of the conditioned cigarettes (see ISO 6488-1:1997).

Average number of puffs per cigarette for each channel to the nearest 0.1 puff.

Total sidestream particulate matter (milligrams per cigarette) for each channel to the nearest 0.l mg and the average per cigarette to the nearest 1 mg.

Dry sidestream particulate matter (milligrams per cigarette) for each channel to the nearest 0.1 mg and the average per cigarette to the nearest 1 mg.

Nicotine-free dry sidestream particulate matter (milligrams per cigarette) for each channel to the nearest 0.1 mg and the average per cigarette to the nearest 1 mg.

Total sidestream nicotine (milligrams per cigarette) for each channel to the nearest 0.01 mg and the average per cigarette to the nearest 0.1 mg.

14. REPEATABILITY AND REPRODUCIBILITY

A major international collaborative study involving 15 laboratories and 7 cigarette samples including the CM3 test piece and spanning a wide range of blends and construction was conducted in 2001 and gave the following values for repeatability (r) and reproducibility (R) of this method.

The difference between two single results found on matched cigarette samples by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability value (r) on average not more than once in 20 cases in the normal and correct operation of the method.

Single results on matched cigarette samples reported by two laboratories will differ by more than the reproducibility (R) on average not more than once in 20 cases in the normal and correct operation of the method.

Data analysis for the 7 cigarettes gave the estimates as summarised in the following table:

Cigarette	Total Sidestream NFDPM (mg/cig)			Total Sidestream nicotine (mg/cig)		
	mean	r	R	mean	r	R
A	13.97	2.66	4.64	4.107	0.578	1.066
B	27.14	3.74	4.89	5.509	0.588	1.072
C	20.40	3.15	5.27	2.879	0.408	0.673
D	22.04	2.96	4.39	5.786	0.745	0.826
E	27.25	3.88	6.15	5.061	0.735	0.768
F	21.58	3.84	5.11	4.996	0.666	1.229
CM3	27.04	3.84	5.44	5.022	0.681	1.042

For the purposes of calculating r and R, one test result was defined as the mean yield obtained from smoking 3 cigarettes in a single run. Eight test results were obtained for each cigarette type by each of the participating laboratories.

14.1. *Confirmation of r and R*

In 2008 the CORESTA Scientific Commission requested that the Routine Analytical Chemistry Sub Group organize a collaborative study to establish the r and R statistics for CM6. In June 2008 the experimental protocol and results template for the collaborative experiment to establish the r and R values for sidestream yields of the CM6 were circulated to the participating laboratories. Eight replicates were requested for each analyte. Twelve laboratories participated in the work and the number of data sets received from laboratories that were able to follow the protocol for each analyte were:

Conditioned weight of the CM6	11
Sidestream (SS) NFDPM yield	12
Sidestream (SS) Nicotine yield	12

Repeatability (r) & reproducibility (R) values were calculated on the remaining data after the exclusion of outliers and these are shown in Table 1 below.

Parameter	No of Labs	Average	Repeatability r	Reproducibility R
Conditioned weight mg/CM6	11	972.49	24.16	37.71
Sidestream NFDPM mg/CM6	11	24.49	3.04	7.62
Sidestream Nicotine mg/CM6	11	4.92	0.56	1.29

The results from this study are comparable to those obtained from the 2002 study.

ANNEX Smoking Plans

(Informative, this Annex does not form an integral part of the Recommended Method)

In the majority of cases the results of mechanical smoking permit a comparison of types of cigarettes (treatments). This comparison should be made according to a smoking plan established in advance. The smoking plan should take account of:

the capacity and the variability of the smoking machine: number of channels;

the capacity of the sidestream smoke collection system: it determines the number of cigarettes to be smoked in each channel;

required precision: the results of smoking always give a certain variability; the distributions of the treatments in each smoking run and of the smoking runs in time should reduce the effects of uncontrolled or badly controlled factors (mechanical or personal); in general the larger the test portion, the greater the precision.

The order of magnitude of the number N of cigarettes in a test portion is fixed for each type as a function of various factors in particular:

the precision sought;

the time necessary for the smoking processes, itself related to the capacity of the machine.

The exact value to be selectedfor N, chosen in the ranges above (see Section **7.1.**) taking into account the preceding factors, is determined by calculation for each experiment taking into account the parameters which characterise it.

Also if

- t denotes the number of types to be compared (treatments);
- s denotes the number of smoking runs to be carried out;
- c denotes the number of channels on the machine;
- q denotes the number of cigarettes smoked into the same sidestream smoke collection system;

then the different parameters are related by the equation

$$t \times N = s \times c \times q$$

The examples of smoking plans proposed below illustrate the preceding remarks. They could correspond to the following objectives:

Example I
Comparison of two types of cigarettes on one single channel smoking machine. The sidestream smoke collection system can collect the sidestream condensate of three cigarettes.

Example II
Comparison of three types of cigarettes on one single channel smoking machine. The sidestream smoke collection system can collect the sidestream condensate of three cigarettes.

Example III
Comparison of two types of cigarettes on one four channel smoking machine. The sidestream smoke collection system can collect the sidestream condensate of three cigarettes.

Example IV
Comparison of five types of cigarettes on one twenty channel smoking machine. The sidestream smoke collection system can collect the sidestream condensate of three cigarettes.

Example I

Comparison of two types of cigarettes on one single channel smoking machine:

- Number of treatments t = 2 (A, B)
- Number of cigarettes in the test sample N = 24
- Number of cigarettes per channel q = 3
- Number of channels c = 1
- Number of smoking runs s = 16 (1, 2, ... 16)

$$2 \times 24 = 16 \times 1 \times 3$$

The number N of cigarettes to be smoked is limited to 24 of each type, so that the duration of the smoking process is not too long. Each smoking run carries only one treatment. Distribute the runs in time while repeating the following sequence four times (k represents successive values 0, 4, 8 and 12) :

Runs	Treatments
1 + k	A
2 + k	B
3 + k	B
4 + k	A

Example II

Comparison of three types of cigarettes on one single channel smoking machine

- Number of treatments t = 3 (A, B, C)
- Number of cigarettes in the test sample N = 24
- Number of cigarettes per channel q = 3
- Number of channels c = 1
- Number of smoking runs s = 24 (1, 2, ... 24)

$$3 \times 24 = 24 \times 1 \times 3$$

Each smoking run carries only one treatment. The runs are distributed in time in an ordered fashion, eg, by means of a matrix of the following type:

B	A	C
C	B	A
A	C	B

Example III

Comparison of two types of cigarettes on one four channel smoking machine

- Number of treatments t = 2 (A, B)
- Number of cigarettes in the test sample N = 24
- Number of cigarettes per channel q = 3
- Number of channels c = 4 (a, b, c, d)
- Number of smoking runs s = 4 (1, 2, 3, 4)

$$2 \times 24 = 4 \times 4 \times 3$$

Allocate the smoking channels in the two treatments utilising the matrix below, which is constructed for four treatments but which is easily adapted to the case of two treatments by identifying A with C on the one hand and B with D on the other. (In general all matrices of dimension g can be utilised for a number of treatments which are sub-multiples of g).

A	B	C	D
D	C	A	B
B	A	D	C
C	D	B	A

Channel Run	a	b	c	d
1	A	B	A	B
2	B	A	A	B
3	B	A	B	A
4	A	B	B	A

In each smoking run, two channels are allocated to each treatment. For example, in run 2:

- cigarette A is smoked in channels b and c
- cigarette B is smoked in channels a and d.

Each type is smoked twice in each of the four channels

Example IV

Comparison of five types of cigarettes on one twenty channel smoking machine

- Number of treatments t = 5 (A, B, C, D, E)
- Number of cigarettes in the test sample N = 24
- Number of cigarettes per channel q = 3
- Number of channels c = 20 (a, b, ... t)
- Number of smoking runs s = 10 (1, 2, ... 10)

5 x 24 = 2 x 20 x 3

Allocate the smoking channels to five treatments using the matrix below:

D	B	E	A	C
A	D	B	C	E
B	A	C	E	D
C	E	D	B	A
E	C	A	D	B

Channel Run	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t
1	D	B	E	A	C	C	E	D	A	B	E	C	B	A	D	B	D	A	C	E
2	A	D	B	C	E	A	C	E	B	D	C	E	A	D	B	A	B	D	E	C

In each smoking run each treatment is smoked in four channels. For example in run 1:

- cigarette A is smoked in the channels d, i, n, r
- cigarette B is smoked in the channels b, j, m, p
- cigarette C is smoked in the channels e, f, l, s
- cigarette D is smoked in the channels a, h, o, q
- cigarette E is smoked in the channels c, g, k, t

Thus each treatment is smoked in 8 different channels.

APPENDIX 1 - FIGURES REFERENCED IN THE TEXT

Figure 1: FISHTAIL CHIMNEY DIMENSIONS

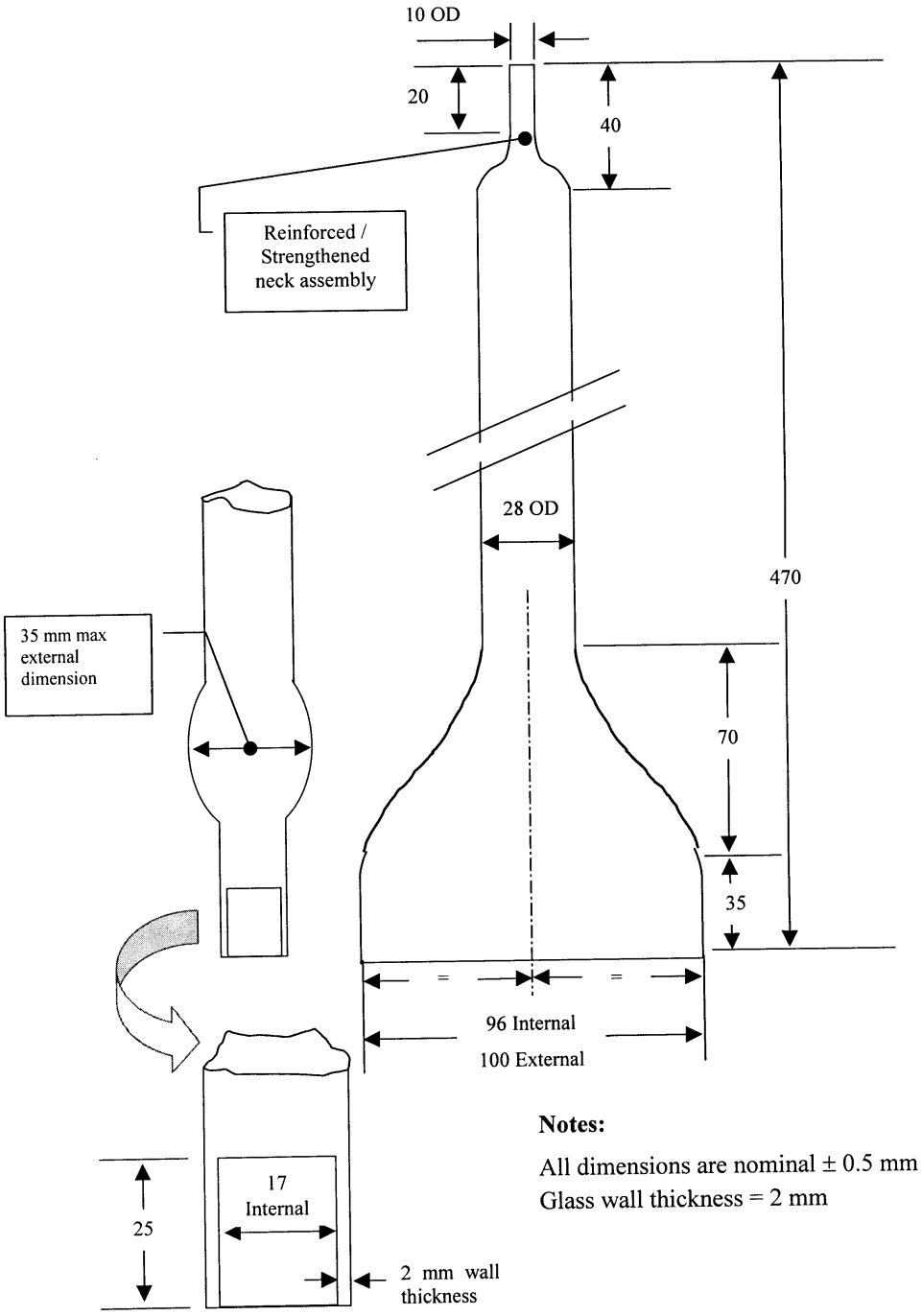
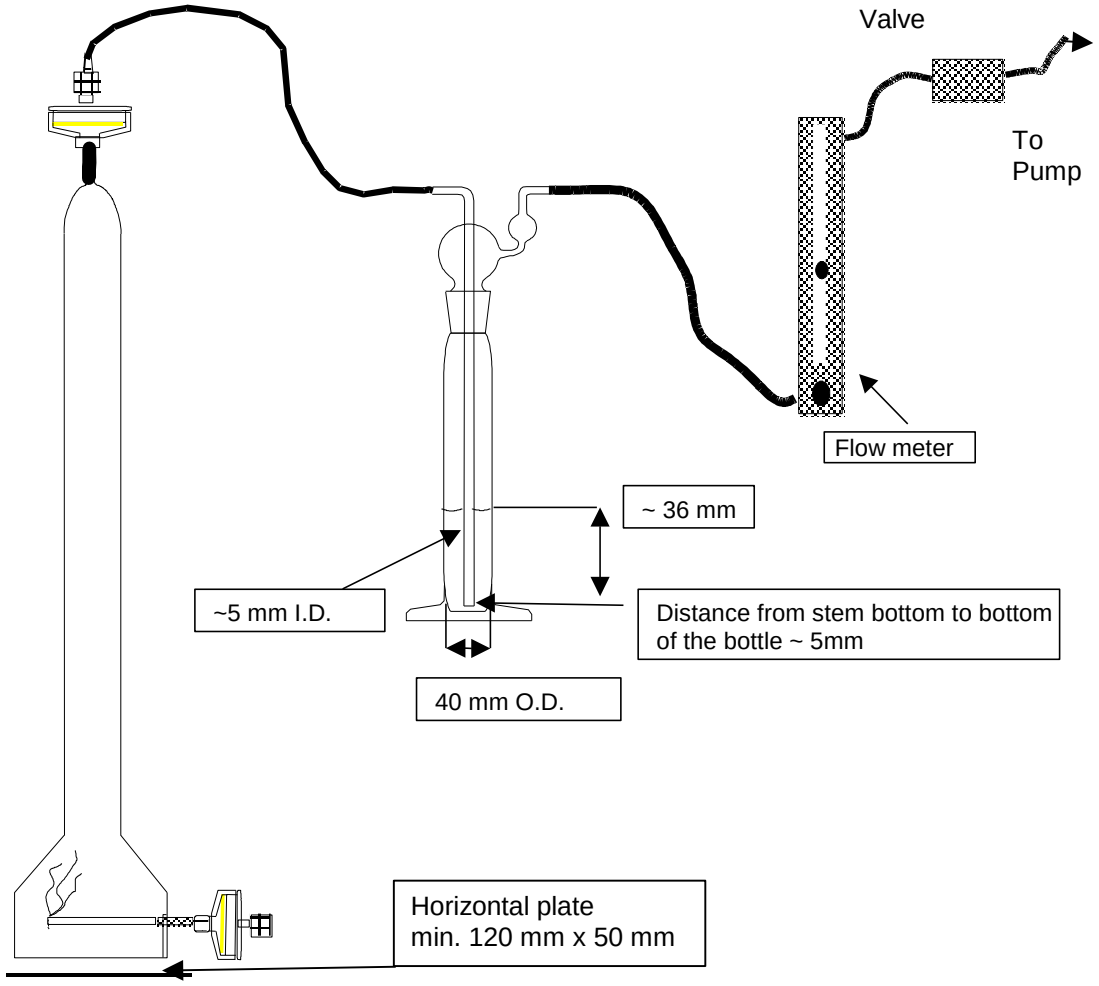


Figure 2: SIDESTREAM SMOKE COLLECTION SYSTEM WITH IMPINGER IN PLACE



APPENDIX 2 - TYPICAL DILUTION FACTORS FOR ESTIMATION OF CHIMNEY PARTICULATE MATTER

Below is a proposed dilution scale for obtaining suitable UV absorbance in the chimney extracts, predicted on the basis of the sidestream pad weight increase. Note that in this table a calculation factor of 1.25 is included to represent the following facts:

(i) that the initial volume of the chimney extract is 50 ml, while that of the sidestream pad extract is 20 ml (50/20 = 2.50)

(ii) that the sidestream pad extract is diluted two-fold in relation to the chimney extract (2.50/2 = 1.25).

SS Pad WTPM (mg)	***Calculation Factor***	***Chimney Dilution***		***SS Pad Dilution***	
		Sample Vol. (ml)	**Solvent Vol (ml)**	**Sample Vol. (ml)**	**Solvent Vol (ml)**
0-1	1.25	No Dilution		8.0	8.0
1.1-10	1.0938	0.9	6.1	0.9	15.1
10.1-25.0	0.5556	0.9	7.1	0.4	15.6
25.1-35.0	0.4688	0.8	7.2	0.3	15.7
>35.0	0.5000	0.5	7.5	0.2	15.8

The calculation factors are worked out as shown in the following example:

Sample SS WTPM in the 1.1 – 10 mg range

Chimney

3 cig/50 ml = x cig/0.9 ml

x = 0.05400000 cig

0.5400000 cig/7.0 ml = 0.00771428 cig/ml

Pad

3 cig/20 ml = x cig/0.9 ml

x = 0.1350000 cig

0.1350000 cig/16.0 ml = 0.00843750 cig/ml

0.00843750 (cig/ml)/0.007714289 (cig/ml) = 1.0938