British American Tobacco Group Research & Development

Method - Determination of nitric oxide in mainstream smoke

1 SCOPE OF APPLICATION

The method is applicable to the quantitative determination of nitric oxide in the vapour phase of the mainstream smoke of cigarettes, using a routine smoking machine and a chemiluminescence detector.

2 NORMATIVE REFERENCES

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

ISO 3402:1999 – Tobacco and tobacco products – atmospheres for conditioning and testing

ISO 4387:2000 – Cigarettes - Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine

ISO 8243:2006 – Cigarettes - Sampling

3 PRINCIPLE

Cigarettes are smoked on a rotary smoking machine. The vapour phase of each puff of smoke is sampled and the concentration of nitric oxide is determined using a chemiluminescence detector. The chemiluminescence signal is obtained by mixing the smoke vapour phase with ozone, which oxidises the nitric oxide to an excited form of nitrogen dioxide. This relaxes to the ground state by emitting light, which is captured and amplified by the photomultiplier to produce the signal used for quantification. The detector is calibrated by means of a range of nitric oxide/nitrogen mixtures.

Puff-by-puff analysis is required because nitric oxide reacts with other smoke components at an appreciable rate. Each puff is therefore analysed immediately after generation. The total nitric oxide delivery of the cigarette is obtained by adding the delivery of nitric oxide from each puff.

4 HEALTH & SAFETY

Read and understand the Material Safety Data Sheets for the chemicals used in this method. Read and understand the method risk assessment. Ensure that you understand the hazards and follow control measures relevant to the operation of this method. All preparation of standards and extraction of samples must be performed in a fume cupboard.

5 REAGENTS AND MATERIALS

Nitrogen gas - ultra high purity

Certified standard gas mixtures containing approximately 100, 200, 500, and 100 parts per million (ppm) of nitric oxide in nitrogen. The gas mixtures are supplied in aluminium cylinders with a certificate of analysis giving the exact composition.

6 APPARATUS

Borgwaldt-KC routine rotary smoking machine equipped with labyrinth holders, seals and washers to accommodate cigarettes of differing diameters

Cambridge filter (CF) 92 mm diameter holder for Borgwaldt-KC smoking machine

Perspex 44 mm Cambridge filter pad holder for protection of syringe and analyser. This is placed in line in between the smoking machine and pneumatic panel.

Cambridge Filter pads, 92 and 44 mm.

A calibrated soap film flow meter, which will measure the puff volume to within ± 0.1 cm^3

Borgwaldt-KC leak detector

Anemometer: Lambrech anemometer model 642 (or equivalent)

Gas divider (Signal 821 SM AK or equivalent)

NO/NO_2 Analyser (ECO PHYSICS or equivalent)

Barometer

Thermometer

PTFE tubing, 5 mm internal diameter

PTFE tubing, 4 mm internal diameter

Stainless Steel 4mm equal T-union

7 PRELIMINARY SAMPLE PREPARATION

Cigarettes should be conditioned according to normal procedures (ISO 3402:1999). Unless specifically requested, cigarettes are not subjected to any selection criteria other than the rejection of any obviously defective or damaged cigarettes. Butt marking is as specified in ISO 4387:2000 unless otherwise requested.

8 ANALYTICAL PROCEDURE –CALIBRATION GAS MIXTURES PREPARATION

8.1 Calibration gas mixtures

Certified nitric oxide in nitrogen mixtures of composition 100, 200, 500, and 1000 ppm nitric oxide are used to enable the measurement of a wide range of nitric oxide levels in cigarette smoke vapour phase. They are diluted as necessary to establish an appropriate calibration range. Dilution is by use of a gas divider.

9 ANALYTICAL PROCEDURE – SAMPLE PREPARATION

9.1 Sample Collection

Ten cigarettes of the test sample are smoked per run, and are placed in alternate ports of a 20-port smoking machine. The smoking machine is equipped with a filter pad holder containing a 92 mm Cambidge filter pad. Pad and holder are weighed before and after smoking to obtain the weight of smoke total particulate matter. The smoke vapour phase passes through the Cambridge filter pad and a second protective filter pad before entering a stainless steel switching valve and a connected T-piece which directs a proportion of the vapour phase to the detector, the remainder being directed to exhaust.

10 ANALYTICAL PROCEDURE – INSTRUMENTAL ANALYSIS

10.1 Instrument Set Up Parameters

Turn on the power switch of the ozone destroyer/pump unit. The vacuum pump will start automatically after a set temperature is reached by the ozone destroyer.

Ensure that the zero gas is flowing. When the pump starts up wait at least 5 minutes to flush all the tubing and components of the analyser with the zero gas.

Turn on the main switch of the analyser, and allow to stabilise for 30 minutes.

The analyser is fully operational when the reaction chamber pressure has reached 120 mbar or less, but should be left for 1 hour to stabilise before calibration.

The normal operating pressure for the internal reaction chamber is 35 mbar (± 10 mbar).

10.2 Detector Calibration

Calibrate the detector using nitrogen to set the zero point and a standard nitric oxide/nitrogen mixture (normally 1000 ppm nitric oxide) to set the span.

Generate a three-point calibration curve by use of the gas divider to produce 2-fold and 10-fold dilutions in nitrogen of the 1000 ppm gas mixture, introducing each of these in turn into the detector. Ensure that the tubing and detector have been thoroughly flushed through with each gas, and that the detector reading is stable, before accepting the value. Custom software is used to calculate the calibration curve.

10.3 System Suitability Criteria

10.3.1 Calibration linearity

The R^2 value of the calibration line must be >0.99.

10.4 Run Order

After calibration is complete, a reference cigarette is smoked and analysed, followed by the test cigarettes. The software records, for each smoke run, the nitric oxide concentration in ppm (parts per million by volume) of each puff taken from each cigarette. The ambient temperature and pressure are recorded.

11 CALCULATIONS

The detector determines the nitric oxide concentration in ppm for each puff. From this, the software calculates the nitric oxide (NO) content in µg per puff using the following formula:

$$NO(\mu g) = \frac{30 \times NO_{ppm} \times v \times 273.16 \times p \times 10^6}{10^6 \times 22.414 \times 10^3 \times (273.16 + t) \times 1013.25}$$

where:

NO [µg]	=	Nitric oxide yield per puff
v [mL]	=	Puff volume (normally 35 mL)
NO [ppm]	=	Nitrogen monoxide concentration per puff in ppm
p [mbar]	=	Atmospheric air pressure
273.16 [°K]	=	0 ^{o}C in Kelvin
22.414 (L)	=	Molar volume of an ideal gas at 0°C and 1 atmosphere pressure
1013.25 [mbar]	=	1 atmosphere
t [°C]	=	Ambient temperature

This equation may be simplified to:

$$NO(\mu g) = \frac{0.00036083 \times NO_{ppm} \times v \times p}{(273.16 + t)}$$

Summation over all the puffs of a cigarette gives the total NO content of a cigarette. This calculation is repeated for each of the 10 cigarettes normally smoked per run.

Finally, the average NO content of all cigarettes smoked is calculated.

12 PRECISION AND REPORTING LIMITS

Five replicate smokings and analyses are performed to determine the precision of the analysis. Longer-term precision is monitored through the maintenance of control charts.

The reporting limit for the method as normally operated is defined as the concentration of the lowest calibration standard (normally 100 ppm nitric oxide). This equates to approximately 5 µg nitric oxide per 35 mL puff. For cigarette types which deliver lower yields of nitric oxide, a lower calibration range may be required, and the reporting limit will change accordingly.

13 QUALITY ASSURANCE AND CONTROL

Control charts of reference cigarettes are maintained to allow inspection of the method performance.

14 SPECIAL CASES

Depending on the nitric oxide delivery of the test cigarettes and the smoking regime employed, the calibration range may need to be altered by appropriate choice of the calibration gas mixture.

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