Disclosure to Promote the Right To Information

Whereas the Parliament of India has set out to provide a practical regime of right to information for citizens to secure access to information under the control of public authorities, in order to promote transparency and accountability in the working of every public authority, and whereas the attached publication of the Bureau of Indian Standards is of particular interest to the public, particularly disadvantaged communities and those engaged in the pursuit of education and knowledge, the attached public safety standard is made available to promote the timely dissemination of this information in an accurate manner to the public.

"जानने का अधिकार, जीने का अधिकार"
Mazdoor Kisan Shakti Sangathan
"The Right to Information, The Right to Live"

"पुराने को छोड़ नये के तरफ"
Jawaharlal Nehru
"Step Out From the Old to the New"

Indian Standard

CIGARETTES — DETERMINATION OF NICOTINE IN SMOKE CONDENSATES — GAS-CHROMATOGRAPHIC METHOD

ICS 65.160
NATIONAL FOREWORD

This Indian Standard which is identical with ISO 10315 : 2000 ‘Cigarettes — Determination of nicotine in smoke condensates — Gas-chromatographic method’ issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Tobacco and Tobacco Products Sectional Committee and approval of the Food and Agriculture Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words ‘International Standard’ appear referring to this standard, they should be read as ‘Indian Standard’.

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard reference appear to the following International Standard for which Indian Standard also exists. The corresponding Indian Standard which is to be substituted in its place is listed below along with its degree of equivalence for the edition indicated:

<table>
<thead>
<tr>
<th>International Standard</th>
<th>Corresponding Indian Standard</th>
<th>Degree of Equivalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine</td>
<td>Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine</td>
<td></td>
</tr>
</tbody>
</table>

The technical committee has reviewed the provision of the following International Standard referred in this adopted standard and has decided that it is acceptable for use in conjunction with this standard:

<table>
<thead>
<tr>
<th>International Standard</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISO 13276</td>
<td>Tobacco and tobacco products — Determination of nicotine purity — Gravimetric method using tungstosilicic acid</td>
</tr>
</tbody>
</table>

Technical Corrigendum 1 published in 2000 to the above International Standard has been given at the end of this publication.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 ‘Rules for rounding off numerical values (revised)’. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.
1 Scope

This International Standard specifies a method for the gas-chromatographic determination of nicotine in cigarette smoke condensates. The smoking of cigarettes and the collection of mainstream smoke are normally carried out in accordance with ISO 4387. However, the method specified in this International Standard is also applicable to the determination of nicotine in cigarette smoke condensates obtained by non-standard smoking.

NOTE In countries not in a position to use the gas-chromatographic method, reference should be made to ISO 3400 for the determination of total nicotine alkaloids. In such cases, values obtained using the method described in ISO 3400 may be used with the addition of a note in the expression of results.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 4387, Cigarettes — Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine.

ISO 13276, Tobacco and tobacco products — Determination of nicotine purity — Gravimetric method using tungstosilicic acid.

3 Principle

The smoke condensate from the mainstream smoke is dissolved in a solvent containing an internal standard. The nicotine content of an aliquot of the solution is determined by gas chromatography, and the nicotine content of the whole of the smoke condensate is calculated.

4 Reagents

Use only reagents of recognized analytical reagent grade.

4.1 Carrier gas: helium or nitrogen of high purity.

4.2 Auxiliary gases: air and hydrogen of high purity for the flame ionization detector.

4.3 Propan-2-ol, with maximum water content of 1.0 mg/ml.

4.4 Internal standard: n-heptadecane or quinaldine (of purity 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. The peak
area of the internal standard on samples should be monitored for consistency. In cases where inconsistencies are
found, analysis of an extraction of a smoke sample without the internal standard in the extraction solution should be
performed to confirm the absence of a peak in the smoke extract eluting at the same time as the internal standard
(see clause 9).

4.5 **Extraction solvent**: propan-2-ol (4.3) containing an appropriate concentration of internal standard (4.4); this
is normally in the range of 0,2 mg/ml to 0,5 mg/ml.

Solvent not stored in a temperature-controlled laboratory shall be allowed to equilibrate to (22 °C ± 2 °C) before use.

4.6 **Reference substance**: nicotine of known purity and verified in accordance with ISO 13276.

Store this at between 0 °C and 4 °C and exclude light.

Nicotine salicylate of known purity and verified in accordance with ISO 13276 may also be used.

4.7 **Calibration solutions**

Dissolve the nicotine (4.6) in the solvent (4.5) to produce a series of at least four calibration solutions whose
concentrations cover the range expected to be found in the test portion (usually 0,02 mg/ml to 2,0 mg/ml). Store
these solutions at between 0 °C and 4 °C and exclude light.

Solvent and solutions stored at low temperatures shall be allowed to equilibrate to (22 °C ± 2 °C) before use.

5 **Apparatus**

Usual laboratory apparatus and, in particular, the following items.

5.1 **Gas-chromatograph**, equipped with a flame ionization detector, recorder and integrator or other suitable
data handling instrument (see clause 9).

5.2 **Column**, of internal diameter between 2 mm and 4 mm and preferably of length 1,5 m to 2 m.

The column is preferably made of glass but other materials such as deactivated stainless steel or nickel may be
used. Stationary phase: 10 % PEG 20 000 plus 2 % potassium hydroxide on an acid-washed silanized support
material, 150 μm (100 mesh) to 190 μm (80 mesh) (see also clause 9).

6 **Procedure**

6.1 **Test portion**

Prepare the test portion by dissolving the smoke condensate obtained by the machine smoking of a known number
of cigarettes in a fixed volume of the solvent (4.5) of 20 ml for 44 mm discs, or 50 ml for 92 mm discs, ensuring that
the disc is fully covered. The volume may be adjusted to give a concentration of nicotine appropriate for the
calibration graph (see 6.3) provided that there is adequate volume for effective extraction of the smoke condensate.
Analysis should be performed as soon as possible but if storage is inevitable then store the sample at between
0 °C and 4 °C and exclude light. For standard smoking, refer to ISO 4387.

6.2 **Setting up the apparatus**

Set up the apparatus and operate the gas chromatograph (5.1) in accordance with the manufacturer's instructions.
Ensure that the peaks for solvent, internal standard, nicotine and other smoke component peaks, especially
nephtadiene (which can appear on the tail of the nicotine peak under certain circumstances), are well resolved (see also clause 9).

Suitable operating conditions are as follows:

- column temperature, 170 °C (isothermal);
- injection temperature, 250 °C;
- detector temperature, 250 °C;
- carrier gas, helium or nitrogen at a flow rate of about 30 ml/min;
- injection volume, 2 μl.

Using the above conditions, the analysis time is about 6 min to 8 min (see also clause 9).

6.3 Calibration of the gas chromatograph

Inject an aliquot (2 μl) of each of the calibration solutions (4.7) into the gas chromatograph. Record the peak areas (or heights) of the nicotine and internal standard (4.4). Carry out the determination at least twice.

Calculate the ratio of the nicotine peak to the internal standard peak from the peak area (or height) data for each of the calibration solutions. Plot the graph of the nicotine concentrations in accordance with the area ratios or calculate a linear regression equation (concentration of nicotine according to the area ratios) from these data. The graph should be linear and the regression line should pass through the origin. Use the slope of the regression equation.

Perform this full calibration procedure daily. In addition, inject an aliquot of an intermediate concentration standard after every 20 sample determinations. If the calculated concentration for this solution differs by more than 3 % from the original value, repeat the full calibration procedure.

6.4 Determination

Inject aliquots (2 μl) of the test portion (6.1) into the gas chromatograph. Calculate the ratio of the nicotine peak/internal standard peak from the peak area (or height) data.

Carry out two determinations on the same test portion (6.1).

Calculate the mean value of the ratio from the two determinations.

Where results are obtained from a number of separate channels of smoking and where an auto-sampler is used, a single aliquot portion from the smoke traps is considered adequate.

7 Expression of results

Calculate the concentration of nicotine in the test portion using the graph or linear regression equation prepared in 6.3. From the concentration of nicotine in the test portion, calculate the amount of nicotine in the smoke condensate. Deduce the amount in the cigarettes smoked. Express the test results in milligrams per cigarette, \( m_N \), for each channel to the nearest 0,01 mg, and the average per cigarette to the nearest 0,1 mg.
8 Repeatability and reproducibility

A major international collaborative study involving 30 laboratories and 6 samples, conducted in 1990, showed that when cigarettes are smoked in accordance with ISO 4387 and the resulting smoke solutions are analysed by this method, the following values for the repeatability limits ($r$) and the reproducibility limits ($R$) are obtained.

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 limit ($r$) 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 limit ($R$) on average not more than one in 20 cases in the normal and correct operation of the method.

Data analysis gave the estimates as summarized in Table 1.

### Table 1 — Estimates given by data analysis

<table>
<thead>
<tr>
<th>Mean value</th>
<th>Repeatability limit</th>
<th>Reproducibility limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_N$ mg per cigarette</td>
<td>$r$ mg per cigarette</td>
<td>$R$ mg per cigarette</td>
</tr>
<tr>
<td>0.091</td>
<td>0.040</td>
<td>0.069</td>
</tr>
<tr>
<td>0.179</td>
<td>0.046</td>
<td>0.069</td>
</tr>
<tr>
<td>0.326</td>
<td>0.050</td>
<td>0.076</td>
</tr>
<tr>
<td>0.673</td>
<td>0.077</td>
<td>0.109</td>
</tr>
<tr>
<td>0.835</td>
<td>0.079</td>
<td>0.142</td>
</tr>
<tr>
<td>1.412</td>
<td>0.107</td>
<td>0.195</td>
</tr>
</tbody>
</table>

For the purpose of calculating $r$ and $R$, one test result was defined as the mean yield obtained from smoking 20 cigarettes in a single run.

For further details of the interaction of $r$ and $R$ with other factors, see CORESTA Report 91/1.

The subject of tolerances due to sampling is dealt with in ISO 8243.

9 Alternative gas chromatographic procedures and analysis precautions

9.1 General

Alternative gas chromatographic columns, both packed and capillary have been found suitable for the determination of nicotine in smoke condensate. If these are used, it is necessary to ensure that the peaks due to nicotine and the internal standard are well resolved from peaks due to other smoke components and the solvent.

The data in clause 8 refer to the reference column; appropriate data for these alternative procedures are not yet available.
9.2 Alternative columns

9.2.1 Packed columns

The following may be used as alternative stationary phases in the column described in 5.2:

— 2 % Versamid 900\textsuperscript{1}) plus 1 % potassium hydroxide, or
— 7 % PEG 20 000 plus 3 % polyphenyl ether (6 rings), or
— lower loadings of PEG 20 000 (with or without potassium hydroxide).

9.2.2 Capillary columns

Fused silica capillary columns (0,2 mm to 0,53 mm ID) with a thin film thickness equal to or less than 1 \textmu m, capable of analysing polar compounds, may be used.

Base-deactivated poly(ethylene glycol) stationary phases such as CAM (J & W Scientific)\textsuperscript{1}), Carbowax-amine (Supelco)\textsuperscript{1}), Stabilowax-DB (Restek)\textsuperscript{1}), and CP WAX-51 (Chrompack)\textsuperscript{1}) give similar data to the PEG 20 000 plus potassium hydroxide packed column in 9.2.1.

9.3 Injection systems

The alternative columns described in 9.2.1 and 9.2.2 require the use of purpose-made injection systems. Suitable operating conditions may vary depending on the type of column used and they may need to be optimized following the manufacturer’s instructions. Isothermal oven temperature or oven temperature programming, hold times, carrier gas and linear velocity and split ratio shall be set for the type of capillary column used. For example; for a 15 m, 0,32 mm ID, 0,25 \textmu m film thickness capillary column, typical conditions might be as follows:

— oven temperature 160 °C (hold 4,5 min) rising to 200 °C at 30 °C/min (hold 1,5 min),
— carrier gas helium at a linear flow rate of about 25 mm/s;
— split ratio 20:1.

Using the above conditions, the analysis time is about 7 min to 8 min.

9.4 Alternative internal standards

Alternative internal standards have also been evaluated. These are carvone, quinaldine and \textit{n}-octadecane. These may be used after assessment of their purity and a check to ensure that they do not co-elute with other smoke components in the smoke extract being analysed. The peak area of the internal standard on samples should be monitored for consistency.

Where inconsistencies are found, analysis of a smoke sample without an internal standard in the extraction solution should be performed to confirm the absence of a peak in the smoke extract eluting at the same time as the internal standard.

\textsuperscript{1}) These are trade names of examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.
10 Test report

The test report shall state the yield of nicotine per cigarette smoked and the method used, and shall include all conditions which may affect the result (e.g. atmospheric test conditions during smoking). It shall also give all details necessary for the identification of the cigarettes smoked.
Annex A
(informative)

Use of this method with the gas-chromatographic determination of water

This method may be used in conjunction with, or simultaneously with, the gas-chromatographic method of water determination in smoke condensates specified in ISO 10362-1. This may be carried out by

— the addition of an appropriate quantity of the internal standard specified for the water determination in the solvent described in 4.5;

— the use of helium, preferably, as the carrier gas;

— injection of an aliquot of the smoke condensate solution onto a column for water analysis, which is connected to a thermal conductivity detector, as well as onto the nicotine column and detector described in this method.

A simultaneous automated analysis of nicotine and water may be achieved by using a splitting system or an autosampler with two injection positions. When determining nicotine and water from the same sample sequentially, the water determination is performed first to prevent absorption of water by the sample affecting the final result.
Bibliography


TECHNICAL CORRIGENDUM 1

Technical Corrigendum 1 to International Standard ISO 10315:2000 was prepared by Technical Committee ISO/TC 126, Tobacco and tobacco products.

Page 5, subclause 9.3

9.3 Injection systems

In the second item of the list: — carrier gas, replace “25 mm/s” by “25 cm/s”.

----------
Bureau of Indian Standards

BIS is a statutory institution established under the Bureau of Indian Standards Act, 1986 to promote harmonious development of the activities of standardization, marking and quality certification of goods and attending to connected matters in the country.

Copyright

BIS has the copyright of all its publications. No part of these publications may be reproduced in any form without the prior permission in writing of BIS. This does not preclude the free use, in course of implementing the standard, of necessary details, such as symbols and sizes, type or grade designations. Enquiries relating to copyright be addressed to the Director (Publications), BIS.

Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of ‘BIS Catalogue’ and ‘Standards: Monthly Additions’.

This Indian Standard has been developed from Doc No.: FAD 4 (1957).

Amendments Issued Since Publication

<table>
<thead>
<tr>
<th>Amendment No.</th>
<th>Date of Issue</th>
<th>Text Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BUREAU OF INDIAN STANDARDS

Headquarters:
Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephone: 2323 0131, 2323 3375, 2323 9402 Website: www.bis.org.in

Regional Offices:

<table>
<thead>
<tr>
<th></th>
<th>Telephone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central</td>
<td>Manak Bhavan, 9 Bahadur Shah Zafar Marg</td>
</tr>
<tr>
<td></td>
<td>NEW DELHI 110002</td>
</tr>
<tr>
<td></td>
<td>2323 7617</td>
</tr>
<tr>
<td></td>
<td>2323 3841</td>
</tr>
<tr>
<td>Eastern</td>
<td>1/14, C.I.T. Scheme VII M, V.I.P. Road, Kankurgachi</td>
</tr>
<tr>
<td></td>
<td>KOLKATA 700054</td>
</tr>
<tr>
<td></td>
<td>2337 8499, 2337 8561</td>
</tr>
<tr>
<td></td>
<td>2337 8626, 2337 9120</td>
</tr>
<tr>
<td>Northern</td>
<td>SCO 335-336, Sector 34-A, CHANDIGARH 160022</td>
</tr>
<tr>
<td></td>
<td>260 3843</td>
</tr>
<tr>
<td></td>
<td>260 9285</td>
</tr>
<tr>
<td>Southern</td>
<td>C.I.T. Campus, IV Cross Road, CHENNAI 600113</td>
</tr>
<tr>
<td></td>
<td>2254 1216, 2254 1442</td>
</tr>
<tr>
<td></td>
<td>2254 2519, 2254 2315</td>
</tr>
<tr>
<td>Western</td>
<td>Manakalaya, E9 MIDC, Marol, Andheri (East)</td>
</tr>
<tr>
<td></td>
<td>MUMBAI 400093</td>
</tr>
<tr>
<td></td>
<td>2832 9295, 2832 7858</td>
</tr>
<tr>
<td></td>
<td>2832 7891, 2832 7892</td>
</tr>
</tbody>
</table>

Branches: AHMEDABAD. BANGALORE. BHOPAL. BHUBANESHWAR. COIMBATORE. DEHRADUN. FARIDABAD. GHAZIABAD. GUWAHATI. HYDERABAD. JAIPUR. KANPUR. LUCKNOW. NAGPUR. PARWANOO. PATNA. PUNE. RAJKOT. THIRUVANATHAPURAM. VISAKHAPATNAM.

Published by BIS, New Delhi