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# INTERNATIONAL STANDARD

# ISO 3400

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## Cigarettes — Determination of alkaloids in smoke condensates — Spectrometric method

*Cigarettes — Détermination des alcaloïdes dans les condensats de fumée —  
Méthode spectrométrique*

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Reference number  
ISO 3400 : 1989 (E)

# Cigarettes — Determination of alkaloids in smoke condensates — Spectrometric method

## 1 Scope

This International Standard specifies a method for the spectrometric determination of alkaloids in cigarette smoke condensates.

The method is applicable to methanolic or iso-propanolic solutions of cigarette smoke condensates.

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards listed below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3308 : 1986, *Cigarettes — Routine analytical cigarette-smoking machine — Definitions and standard conditions.*

ISO 3401 : — <sup>1)</sup>, *Cigarettes — Determination of alkaloid retention by the filters.*

ISO 4387 : 1987, *Cigarettes — Determination of total and dry particulate matter using a routine analytical cigarette-smoking machine — Glass fibre filter smoke trap method.*

ISO 8243 : 1988, *Cigarettes — Sampling.*

ISO 8453 : 1987, *Cigarettes — Determination of total and dry particulate matter using a routine analytical cigarette-smoking machine — Electrostatic smoke trap method.*

## 3 Principle

Steam distillation of an aliquot portion of the methanolic or iso-propanolic solution of a cigarette smoke condensate in two steps. Acidification of the solution with a mineral acid and

removal of neutral and acid steam-volatile substances by distillation followed by making the same solution strongly alkaline and distillation of the alkaloids. Spectrometric measurement of the absorbance of the distillate from the alkaline distillation and calculation of the alkaloid content expressed as nicotine.

## 4 Reagents

Use only reagents of recognized analytical grade and distilled water or water of at least equivalent purity.

**4.1 Sodium hydroxide**, solution,  $c(\text{NaOH}) = 8 \text{ mol/l}$ .

**4.2 Sulfuric acid**, solution,  $c(\text{H}_2\text{SO}_4) = 1 \text{ mol/l}$ .

**4.3 Sulfuric acid**, solution,  $c(\text{H}_2\text{SO}_4) = 0,025 \text{ mol/l}$ .

**4.4 Nicotine**, minimum purity 98 %.

## 5 Apparatus

Usual laboratory apparatus and the following items:

**5.1 Steam distillation apparatus**, as described in ISO 3401 or any other apparatus giving the same results.

Test the system according to the indicated procedure (7) with the pure nicotine solution (4.4) at the maximum expected level. Recovery shall be at least 98 % of the theoretical value. If not, optimize by modification of the distillation rate.

For routine tests it is possible to use nicotine salt calibrated against the pure nicotine (4.4).

**5.2 Spectrometer**, covering a wavelength range from 230 nm to 290 nm.

1) To be published.

$l$  is the optical path length of the cell, in centimetres;

$V_0$  is the volume of the methanolic or iso-propanolic solution of crude smoke condensate, in millilitres;

$V_1$  is the aliquot portion of  $V_0$  used for the distillation, in millilitres;

$V_2$  is the volume of distillate from the alkaline distillation, in millilitres;

$V_3$  is the aliquot portion of distillate  $V_2$  used for further dilution to  $V_4$ , in millilitres;

$V_4$  is the volume to which the aliquot portion  $V_3$  of the distillate was further diluted, in millilitres;

$n$  is the number of cigarettes smoked into the smoking trap.

### 8.1.2 Mean alkaloid content of the smoke condensate per set of smoking runs

Calculate the mean of the results obtained for each trap per set of smoking runs.

### 8.1.3 Mean alkaloid content of the smoke condensate for the whole test sample

Calculate the mean of the results obtained for each smoking run.

## 8.2 Expression of results

Express the test results as follows:

a) alkaloid content, expressed as nicotine, in milligrams per cigarette smoked, to the nearest 0,01 mg for each individual smoking run;

b) alkaloid content, expressed as nicotine, in milligrams per cigarette smoked, to the nearest 0,01 mg for each set of smoking runs;

c) mean alkaloid content, expressed as nicotine, in milligrams per cigarette smoked, to the nearest 0,1 mg for the whole test sample or to the nearest 0,01 mg if the confidence interval is expressed.

## 9 Test report

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

The test report shall include all details required for complete identification of the sample.

The test report shall include the items of information listed in 9.2 to 9.5.

9.2 Description of the product tested.

9.3 Sampling procedure:

- a) method of sampling;
- b) number of cigarettes of the test sample;
- c) date and place of purchase or sampling.

9.4 Test results, together with their precision, expressed in accordance with 8.2.

9.5 Date of test and reference to this International Standard.

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