

## CORESTA RECOMMENDED METHOD N° 39

### DETERMINATION OF THE PURITY OF NICOTINE AND NICOTINE SALTS BY GRAVIMETRIC ANALYSIS - TUNGSTOSILICIC ACID METHOD

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#### 0. INTRODUCTION

Several methods for checking the purity of nicotine used to calibrate analytical methods have been suggested since the 1920's. At that time the gravimetric method using picric acid was considered the most specific method while that employing tungstosilicic acid was considered most sensitive. However, neither method could be considered completely specific. Since the 1960's, the most commonly used methods are those employing UV-spectrometry and gravimetry using tungstosilicic acid reagents.

In the late 1980's work within the UK tobacco industry showed that spectrometers with band widths greater than 2 nm were not satisfactory for this determination. They also found that the results for instruments with band widths of less than 2 nm gave low results for pure nicotine when the Willits factor (see note 1) was used. Furthermore, in countries where laboratory accreditation is operated the examiners normally specify that calibration standards shall be traceable to a primary standard. Thus gravimetric methods comply with the accreditation requirements. The described method reports the UK industry's improved version of the AOAC's official method (14<sup>th</sup> edition, 1984) for the determination of nicotine purity by gravimetric analysis using the tungstosilicic acid reagent.

CORESTA studies during 1992 and 1993, involving 19 laboratories, have shown that this modified tungstosilicic acid method gives very consistent results for the purity of samples of nicotine, nicotine hydrogen tartrate and nicotine salicylate. However, there remained some doubts as to the specificity of the method. In particular, the literature suggests that other alkaloids and degradation products of nicotine may interfere with this determination. In 1993, CORESTA studied the effect of various storage conditions and their influence on the degradation of nicotine (GC purity > 99% by mass) and the subsequent influence of this degradation on the determination of nicotine by the tungstosilicic acid, gas chromatographic and UV-spectrometric methods. These studies revealed:

- high purity nicotine degrades slowly. For example, storage of nicotine (GC purity > 99% by mass) in a refrigerator for 18 months in the presence of air reduces the purity to about 97%. A similar decrease in purity is observed during 2 months storage at room temperature in the presence of light and air.
- water was often found to be a major contaminant after storage and may be present at the % level. In theory all methods detect this contamination satisfactorily.
- GC/MS investigations revealed that the major degradation products after storage of pure nicotine are cotinine and myosmine. The maximum levels of these observed in the tests were 1%.

- Both these observed degradation products will influence, to some extent, the purity determined by all methods except that for GC/MS. For the tungstosilicic acid and the UV-spectrometric methods this interference was shown to be equivalent and small.
- In practice, the tungstosilicic acid results were found to be equivalent to the purity determined by GC/MS analysis after correction for water content *i.e.* best estimate for samples with purity greater than 96% nicotine.
- On the other hand, UV-spectrometric results were found to be equivalent or slightly lower than the 'best estimate' value. This gave some support to the UK industry's findings.

The CORESTA studies on the tungstosilicic acid method indicate that the most practical and appropriate method for the determination of nicotine purity for samples containing at least 96% nicotine is the described tungstosilicic acid method. Interference due to degradation products at this purity level is negligible.

Initial CORESTA studies in 1992 showed that although the tungstosilicic acid method could determine the purity of nicotine tartrate and salicylate precisely, some samples appeared to have a purity greater than 100%. Further analysis by gas chromatography and UV-spectrometry confirmed that the available nicotine from these samples was indeed greater than that for 100% pure compounds. All the evidence available to CORESTA indicates that these batches of salts are contaminated with unreacted nicotine. Thus for calibration purposes these apparently anomalous results are in fact correct. For salts, the tungstosilicic acid method thus determines the available nicotine rather than the purity of the salt.

Since the salts are stable, the influence of storage on the traces of unreacted nicotine will be even less important than for the pure nicotine itself. The tungstosilicic acid method is thus applicable to the salts.

**Note 1 :** WILLITS, C.O., *et al.* ANAL. CHEM., **22**, 430 (1950).

## 1. PRINCIPLE

The nicotine alkaloid is complexed with dodeca-tungstosilicic acid (silicotungstic acid) to form insoluble nicotine silicotungstate. The precipitate weight is determined either by filtration on to an ashless filter paper or into a sintered glass filter (Gooch crucible).

## 2. REAGENTS

### 2.1 *Dodeca-Tungstosilicic Acid Solution (SiO<sub>2</sub>12WO<sub>3</sub>·26H<sub>2</sub>O)*

Dissolve 12 g dodeca-tungstosilicic acid in 100 cm<sup>3</sup> distilled water.

**Note 2 :** Avoid the use of the other forms of tungstosilicic acid such as 4H<sub>2</sub>O·SiO<sub>2</sub>10WO<sub>3</sub>·3H<sub>2</sub>O or 4H<sub>2</sub>O·SiO<sub>2</sub>12WO<sub>3</sub>·20H<sub>2</sub>O as they do not yield crystalline precipitate with nicotine.

### 2.2 *Hydrochloric Acid Solution (HCl, 20% V/V)*

Dilute 20 cm<sup>3</sup> of hydrochloric acid, p.a. (specific gravity 1.18) to 100 cm<sup>3</sup> with distilled water.

**2.3** *Hydrochloric Acid Solution (HCl, 0.1 % V/V)*

Dilute 5 cm<sup>3</sup> of 20% hydrochloric acid solution (2.2) to 1 dm<sup>3</sup> with distilled water.

**2.4** *Nicotine solution (C<sub>10</sub>H<sub>14</sub>N<sub>2</sub>)*

Prepare, by dilution with distilled water, 25 cm<sup>3</sup> of a nicotine solution with a concentration of approximately 0.1 mg nicotine per cm<sup>3</sup>.

### 3. APPARATUS

**3.1** The necessary general laboratory equipment for the preparation of samples, standards and reagents.

**3.2** Laboratory oven capable of attaining 120 ± 5°C for the glass filter procedure (4.4.4) or :

Bunsen burner or furnace capable of attaining more than 600°C for the filter paper procedure (4.4.7).

**3.3** Sintered glass filter (Gooch type) of porosity 2 (40-100 μm) for the glass filter procedure (4.4.1) or :

Ashless filter papers, Whatman N° 42 (or equivalent) for the filter paper procedure (4.4.5).

**3.4** Porcelain or platinum crucibles for the filter paper procedure (4.4.6).

### 4. PROCEDURE

**4.1** Weigh, to the nearest 0.001 g, approximately 0.1 g of the nicotine alkaloid (or the equivalent amount of nicotine salt) in each of five 250 cm<sup>3</sup> beakers equipped with glass stirring rods.

**4.2** Add 100 cm<sup>3</sup> of distilled water to each beaker. Add 2 cm<sup>3</sup> of 20% hydrochloric acid (2.2) to each beaker and stir. Do not remove the stirring rod.

**4.3** Add slowly 15 cm<sup>3</sup> of the tungstosilicic acid solution (2.1) while stirring constantly during the addition. Cover each beaker with a watch glass leaving the stirring rod in place and stand overnight. Before filtering, stir the precipitate to ensure that it settles quickly and is of a crystalline form. Check for complete precipitation with a few extra drops of the tungstosilicic acid solution (2.1).

**4.4** Filtration can be performed following either of the two procedures:

#### **Glass Filter Procedure**

**4.4.1** Dry each sintered glass filter (3.3) in a laboratory oven (3.2) at 120°C until constant weight (± 1 mg). Store in a desiccator.

**4.4.2** Weigh, to the nearest 0.0001 g, each sintered glass filter and filter the precipitate directly into the glass filter using a Buchner flask and vacuum source. Ensure that the precipitate is removed from the sides of the beaker and the stirring rod by washing with 0.1% hydrochloric acid (2,3) (approximately 3 times 15 cm<sup>3</sup>) and discard the washings.

- 4.4.3** Rinse the precipitate with a further aliquot of 0.1 % hydrochloric acid (2.3) (up to 400 cm<sup>3</sup> may be required) which should be collected and tested with a few drops of nicotine solution (2.4) to ensure that no opalescence occurs, *i.e.* all tungstosilicic acid has been removed.
- 4.4.4** Dry each sintered glass filter and precipitate in a laboratory oven (3.2) for 3 hours, cool in a desiccator and weigh, to the nearest 0.0001 g. Place the filters back in the oven for 1 hour, cool and reweigh. Repeat if necessary until a constant weight ( $\pm 1$  mg) is recorded.

### **Filter Paper Procedure**

- 4.4.5** Filter the precipitate directly on to an ashless filter paper (3.3). Ensure that the precipitate is removed from the sides of the beaker and the stirring rod by washing with 0.1% hydrochloric acid (2.3) (approximately 3 times 15 cm<sup>3</sup>) and discard the washings.
- 4.4.6** Rinse with a further aliquot of 0.1% hydrochloric acid (2.3) (up to 400 cm<sup>3</sup> may be required) which should be collected and tested with a few drops of nicotine solution (2.4) to ensure that no opalescence occurs, *i.e.* all tungstosilicic acid has been removed.
- 4.4.7** Weigh, to the nearest 0.0001 g, each crucible (3.4). Transfer the filter paper with the precipitate (4.4.6) to the crucible. Place the crucible on a silica triangle resting on a tripod, heat gently at first and then ignite with a gas or an electric Bunsen burner (3.3). The crucible contents may have to be broken up very carefully to ensure complete removal of the carbon. The final residue should be greenish/yellow in colour. Cool in a desiccator and reweigh to the nearest 0.0001 g. Repeat the heating process until a constant weight ( $\pm 1$  mg) is obtained.

**Note 3 :** After ignition of the paper filter it may be convenient to leave the crucible in a furnace at above 600°C overnight. This technique ensures no further heating is required.

## **5. CALCULATION**

Calculate the nicotine purity using the formula:

$$\% \text{ Nicotine Purity} = \frac{\mathbf{P} \times \mathbf{C}}{\mathbf{m}} \times \mathbf{100}$$

- P** is the mass, in milligrams, of the precipitate (4.4.4 or 4.4.7);
- C** is a constant depending on the filtration procedure  
~ 0.1012 for the glass filter procedure  
~ 0.1141 for the filter paper procedure
- m** is the mass, in milligrams, of the sample (4.1).

The test result shall be the mean of the 5 determinations, expressed to one decimal place.

## 6. REPEATABILITY AND REPRODUCIBILITY

An international collaborative study involving 17 laboratories and 2 samples conducted in 1993 showed that when pure nicotine and a degraded sample of the pure nicotine were analyzed by this method, the following values for repeatability (r) and reproducibility (R) were obtained.

The difference between two test results found on different analyses by one operator using the same apparatus within a short 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 test results reported by two laboratories will differ by more than the reproducibility value (R) on average not more than once in 20 cases in the normal and correct operation of the method.

Data analysis gave the estimates as summarized in the table.

TABLE

Nicotine Type	Mean Purity of Nicotine %	Repeatability Conditions r	Reproducibility Conditions R
Pure (>99%)	98.8	2.2	3.8
Degraded	96.7	1.6	3.2

For the purpose of calculating r and R, one test result was defined as the yield obtained from analyzing one sample once.

**Note 4 :** Estimation of R based on average of 5 single determinations gave the following values: 2.9 (degraded nicotine) and 3.3 (pure nicotine).