



**Cooperation Centre for Scientific Research
Relative to Tobacco**

Routine Analytical Chemistry Sub-Group

**CORESTA Recommended Method
No. 85**

**TOBACCO -
DETERMINATION OF THE
CONTENT OF TOTAL ALKALOIDS
AS NICOTINE -
Continuous Flow Analysis Method
Using KSCN/DCIC**

April 2017



CORESTA RECOMMENDED METHOD N° 85

Title:

**TOBACCO — DETERMINATION OF THE CONTENT OF TOTAL ALKALOIDS AS
NICOTINE — CONTINUOUS-FLOW ANALYSIS METHOD USING KSCN/DCIC**

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TOBACCO — DETERMINATION OF THE CONTENT OF TOTAL ALKALOIDS AS NICOTINE — CONTINUOUS-FLOW ANALYSIS METHOD USING KSCN/DCIC

(April 2017)

0. INTRODUCTION

In 2014 the CORESTA Routine Analytical Chemistry Sub-Group (RAC) undertook a collaborative study of two methods for the determination of total alkaloids in tobacco (as nicotine) by segmented continuous-flow analysis. The two methods are CRM No. 35 (CRM35) (basis for ISO 15152) and a new method proposed by China National Tobacco Quality Supervision & Test Center. In CRM35 cyanogen chloride is generated *in situ* by the reaction of potassium cyanide and chloramine T. The proposed method eliminates the use of the potassium cyanide (KCN) by employing potassium thiocyanate (KSCN) with sodium dichloroisocyanurate dihydrate (DCIC) for colour development. Each method was tested using water extracted tobacco and 5 % acetic acid extracted tobacco. Calibration standards were prepared with the same extraction solutions.

1. FIELD OF APPLICATION

This CRM specifies a method for the determination of the content of total alkaloids as nicotine in tobacco by continuous-flow analysis.

This method is applicable to leaf samples, stems, reconstituted tobacco sheet materials and tobacco blends.

2. NORMATIVE REFERENCES

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

ISO 3696, *Water for analytical laboratory use -- Specification and test methods*

3. PRINCIPLE

An aqueous extract (see below) of the tobacco is prepared and the total alkaloids content (as nicotine) of the extract is measured by reaction of sodium citrate and cyanogen chloride. Cyanogen chloride is produced *in situ* by reaction of KSCN and DCIC. The developed brown colour is measured at 460 nm.

Collaborative studies¹ have shown that the method gives equivalent results for water and 5 % acetic acid extracts. It is recommended that 5 % acetic acid extracts should be used if total alkaloids (as nicotine) and reducing substances (see ISO 15153) or reducing carbohydrates (see ISO 15154) are to be carried out simultaneously.

¹ Routine Analytical Chemistry Sub-Group Technical Report, 2014 Collaborative Study Comparing CRM35 for the Determination of Total Alkaloids (as Nicotine) in Tobacco by Continuous Flow Analysis to a New Method with Safer Chemistry – Project 52

4. REAGENTS

Use only reagents of recognized analytical grade. All reagents shall be used according to good laboratory practice and existing national regulations. Water must be high quality distilled or deionized (DI) water, free from organic contamination, e.g. Grade 1 as defined in ISO 3696.

- 4.1 Polyoxyethylene lauryl ether (Brij-35™, 30 % w/w solution), CAS # 9002-92-0
- 4.2 Sodium phosphate dibasic dodecahydrate, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, CAS # 10039-32-4
- 4.3 Sodium phosphate monobasic dihydrate, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, CAS # 13472-35-0
- 4.4 Sodium citrate dihydrate, $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$, CAS # 6132-04-3
- 4.5 Sulphanilic acid, $\text{NH}_2\text{C}_6\text{H}_4\text{SO}_3\text{H}$, CAS # 121-57-3
- 4.6 Potassium thiocyanate (KSCN), CAS # 333-20-0
- 4.7 Sodium dichloroisocyanurate (DCIC), $\text{C}_3\text{Cl}_2\text{N}_3\text{NaO}_3$, CAS # 51580-86-0
- 4.8 Sodium carbonate, Na_2CO_3 , CAS # 497-19-8
- 4.9 Iron(II) sulphate heptahydrate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, CAS # 7782-63-0
- 4.10 Citric acid monohydrate, $\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}$, CAS # 5949-29-1
- 4.11 Nicotine hydrogen tartrate, $\text{C}_{10}\text{H}_{14}\text{N}_2(\text{C}_4\text{H}_6\text{O}_6) \cdot 2\text{H}_2\text{O}$, CAS # 6019-06-3

5. PREPARATION OF SOLUTIONS

Use distilled or deionized water, free from organic contamination, e.g. Grade 1 as defined in ISO 3696. To reach the performance levels stated, reagents and sampler wash must be free of solids and dissolved air.

For best results vacuum filter all reagents through a 0.45 μm filter² (see Figure 1). If necessary, vacuum filter all DI water used in the preparation of standards and for the sampler wash, otherwise degas the water in another way.



Figure 1: Example vacuum filter set-up

² Millipore XX1604700 | MilliSolve Kit, complete with 2L flask is an example of a suitable product available commercially. This information is given for the convenience of the users of this recommended method and does not constitute an endorsement by CORESTA of this product.

5.1 System wash solution

Add 1 mL of Brij-35, 30% solution to about 800 mL DI water and mix. Then dilute to 1000 mL with DI water. Do not store the solution longer than a week and use a clean bottle for the fresh solution.

5.2 Sampler wash solution

Use the extraction solution, DI water or 5% acetic acid as sampler wash solution.

5.3 Potassium thiocyanate solution

Dissolve 2.88 g of potassium thiocyanate in DI water. Dilute to 250 mL with DI water and mix well.

5.4 Sodium dichloroisocyanurate (DCIC) solution

Dissolve 2.20 g of sodium dichloroisocyanurate and dilute to 250 mL with DI water. Prepare a fresh solution each day of measurement. Neutralisation solution A

Dissolve 1 g of citric acid monohydrate and 10 g of ferrous sulfate in about 500 mL of DI water. Dilute to 1000 mL with DI water and mix well.

5.5 Neutralisation solution B

Dissolve 10 g of sodium carbonate in about 500 mL of DI water. Dilute to 1000 mL with DI water and mix well.

5.6 Buffer solution A

Dissolve 71.6 g of sodium phosphate dibasic dodecahydrate and 11.76 g of sodium citrate dihydrate in about 500 mL of DI water. Dilute to 1000 mL with DI water, add 1 mL of Brij-35, 30% solution mix thoroughly.

5.7 Buffer solution B

Dissolve 71.6 g of sodium phosphate dibasic dodecahydrate, 6.2 g of sodium phosphate monobasic dihydrate, 11.76 g of sodium citrate dihydrate and 7.0 g of sulphanilic acid in about 800 mL of DI water. Dilute to 1000 mL with DI water, add 1 mL of Brij-35, 30% solution mix thoroughly.

6. PREPARATION OF STANDARDS

Check the purity of the nicotine hydrogen tartrate according to ISO 13276. The method can also be standardized by using nicotine or other nicotine salts of known purity. In this case, an amount equivalent to the above used nicotine hydrogen tartrate should be used.

6.1 Nicotine stock solution

Weigh 3.75 g (to the nearest 0.0001 g) of nicotine hydrogen tartrate in DI water and dilute to 500 mL in a volumetric flask. The solution contains approximately 2.5 mg nicotine per mL. Store in a refrigerator (0 – 4) °C. Prepare a fresh solution every month.

6.2 Working standards

From the nicotine stock solution and extraction solution (water or 5 % acetic acid solution), prepare a series of at least 5 calibration solutions according to the nicotine concentration which is expected to be found in the test samples (e.g. 0.5%~15% (w/w)). Calculate the exact concentration for each standard taking into account the purity of the nicotine hydrogen tartrate. Store in a refrigerator at (0 – 4) °C. Prepare fresh solutions every two weeks.

7. APPARATUS

The laboratory needs the usual laboratory apparatus and, in particular, the following items.

7.1 Continuous-flow analyser, consisting of

- Autosampler
- Peristaltic pump
- Chemistry manifold with dialyser and delay coils
- Photometric detector equipped with a 460 nm filter
- Data acquisition system or recorder

See Annex A for examples of suitable flow diagrams.

8. PROCEDURE

8.1 Preparation of samples for analysis

Prepare the tobacco for analysis by grinding (the sample should totally pass a 1 mm sieve) and determine the moisture content. If the tobacco is too wet for grinding it can be dried at a temperature not exceeding 40 °C.

8.2 Test portion

Weigh to the nearest 0.1 mg, approximately 250 mg, of the ground tobacco into a 50 mL conical flask. Add 25 mL of the extraction solution (water or 5 % acetic acid solution). Stopper and shake for 30 minutes at > 150 rpm.

8.3 Preparation of test extract

Filter the extract through a quantitative filter paper such as Whatman No 40³ (or equivalent ashless, quantitative filter paper) filter paper, rejecting the first few mL of the filtrate, then collect the filtrate.

Run the sample and standards through the system in the normal manner (e.g. priming with 6 tobacco extracts, calibration standards and samples with 1 intermediate calibration solution after every 6 samples.). If sample concentration lies outside the range of the standards, the sample shall be diluted and run again.

When using 5 % acetic acid extracts, the wash solution shall be 5 % acetic acid.

NOTE: If this method is performed simultaneously with the methods described in ISO 15154 or ISO 15517, combined standards may be prepared. Combined stock solutions may precipitate after about two weeks.

³ Whatman No. 40 is an example of a suitable product available commercially. This information is given for the convenience of the users of this recommended method and does not constitute an endorsement by CORESTA of this product.

9. CALCULATION

- 9.1 Plot a graph of peak height against equivalent nicotine concentration for all of the calibration solutions.
- 9.2 Calculate the percentage of nicotine, w , on a dry weight basis, in the tobacco using the formula

$$w = \frac{c \times V \times 100}{m} \times \frac{100}{100 - M}$$

where

c is the nicotine concentration, expressed in milligrams per millilitre, obtained from the calibration curve (8.3);

V is the volume, in millilitres, of the sample (see 8.2), normally 25 mL;

m is the mass, in milligrams, of the sample (see 8.2);

M the moisture content, expressed as percentage by mass, of the tobacco (see 8.1)

The test result shall be expressed to two decimal places.

10. REPEATABILITY AND REPRODUCIBILITY

In 2014 an international collaborative study⁴ involving 19 laboratories and eight samples (four straight grade tobaccos, a fire-cured cigarette, a blended cigarette, CM7, and 3R4F) was conducted. The repeatability limit (r) and reproducibility limit (R) were calculated for this new KSCN/DCIC method and CRM35 using both water and 5 % acetic acid extractions (see Tables 1 & 2).

The difference between two single results, found on different extractions by one operator using the same apparatus within a short time interval (the time it takes to analyse ~ 40 sample cups) and without recalibration of the equipment during the time of analysis, 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 reported by two laboratories will differ no more than the reproducibility limit (R) on average not more than once in 20 cases in the normal and correct operation of the method.

⁴ Routine Analytical Chemistry Sub-Group Technical Report, 2014 Collaborative Study Comparing CRM35 for the Determination of Total Alkaloids (as Nicotine) in Tobacco by Continuous Flow Analysis to a New Method with Safer Chemistry – Project 52

Table 1 – Extraction with Water

Tobacco Type	Mean Content of Nicotine (% dry weight)		Repeatability		rCV^*		Reproducibility		RCV^*	
	CRM35	KSCN/DCIC	CRM35	KSCN/DCIC	ISO 15152	KSCN/DCIC	CRM35	KSCN/DCIC	CRM35	KSCN/DCIC
Fire-cured	1.76	1.81	0.05	0.04	2.87	2.37	0.12	0.27	6.67	14.81
Burley	4.77	4.77	0.11	0.11	2.35	2.23	0.61	0.52	12.76	10.97
Oriental	0.98	0.98	0.04	0.03	4.07	3.51	0.24	0.19	24.1	19.21
Dark sun-cured	3.71	3.71	0.08	0.06	2.19	1.73	0.48	0.45	12.89	12.02
Fire-cured cigarette	2.11	2.07	0.06	0.04	2.71	2.15	0.34	0.3	15.9	14.33
Blended cigarette	2.02	2.07	0.03	0.03	1.59	1.51	0.2	0.29	9.65	13.86
CM7	2.2	2.22	0.05	0.05	2.41	2.29	0.28	0.3	12.48	13.43
3R4F	2.15	2.12	0.06	0.06	2.9	2.61	0.29	0.24	13.7	11.52

* rCV is $r/\text{mean} \times 100\%$ and RCV is $R/\text{mean} \times 100\%$

Table 2 – Extraction with 5% Acetic Acid

Tobacco Type	Mean Content of Nicotine (% dry weight)		Repeatability		rCV^*		Reproducibility		RCV^*	
	CRM35	KSCN/DCIC	CRM35	KSCN/DCIC	ISO 15152	KSCN/DCIC	CRM35	KSCN/DCIC	CRM35	KSCN/DCIC
Fire-cured	1.74	1.76	0.04	0.04	2.37	2.28	0.13	0.19	7.51	11.04
Burley	4.53	4.53	0.11	0.08	2.23	1.87	0.41	0.56	8.99	12.35
Oriental	0.96	1	0.04	0.03	3.51	3.38	0.14	0.14	14.66	13.7
Dark sun-cured	3.51	3.6	0.07	0.06	1.73	1.6	0.26	0.44	7.46	12.29
Fire-cured cigarette	2.04	2.05	0.05	0.04	2.15	1.79	0.18	0.27	8.69	13.19
Blended cigarette	1.98	2.02	0.04	0.03	1.51	1.59	0.18	0.23	8.99	11.23
CM7	2.11	2.17	0.05	0.04	2.29	1.95	0.17	0.27	8.19	12.25
3R4F	2.08	2.1	0.06	0.05	2.61	2.34	0.26	0.25	12.55	11.52

11. BIBLIOGRAPHY

ISO 15152, *Tobacco — Determination of the content of total alkaloids as nicotine — Continuous-flow analysis method*

CRM No. 35, *Determination of Total Alkaloids (as Nicotine) in Tobacco by Continuous Flow Analysis*

APPENDIX A - SUITABLE FLOW DIAGRAMS

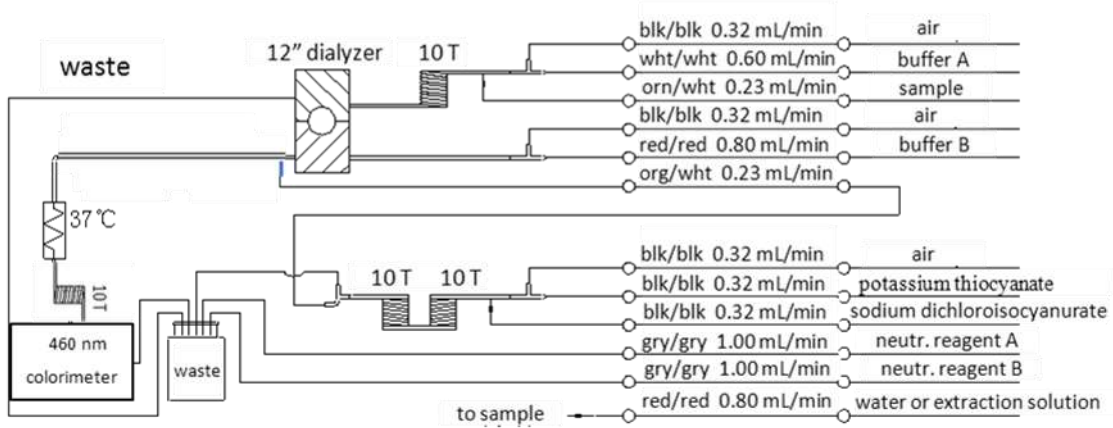


Figure 1: Suggested Flowchart for Macro Flow (mL/min) Systems

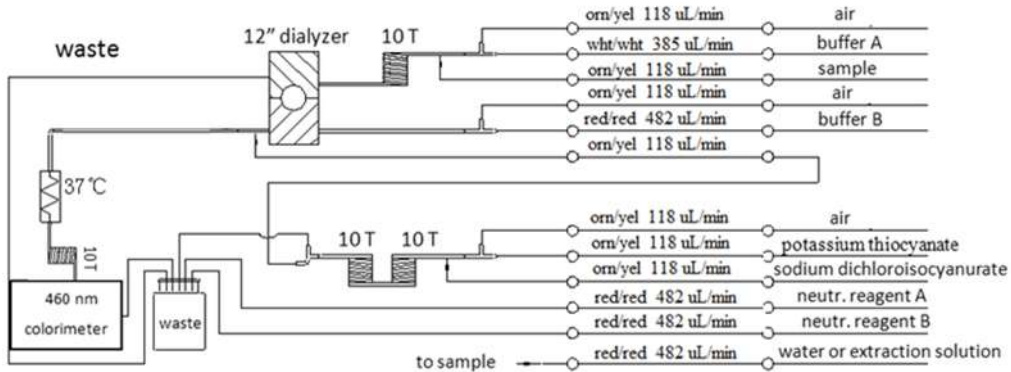


Figure 2: Suggested Flowchart for Micro Flow (µL/min) Systems