

# Methodology



## Nicotine in Tobacco by Segmented Flow Analysis (SFA)

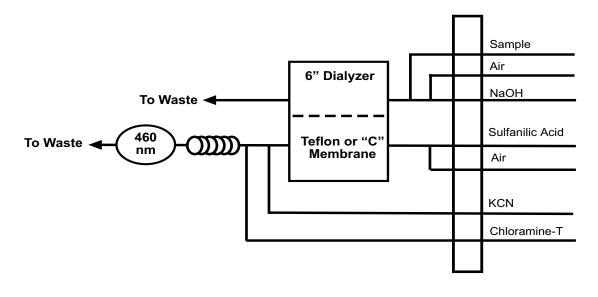
### (Cartridge Part #A002714)

#### **1.0** Scope and Application

- 1.1 This method is used for the determination of nicotine in tobacco abstracts.
- 1.2 The Method Detection Limit (MDL) of this method is 1.0 mg/L. The applicable range of the method is 4.0–200 mg/L. The range may be extended to analyze higher concentrations by sample dilution.

#### 2.0 Summary of Method

- 2.1 Nicotine is reacted with buffered aniline and cyanogen chloride to form a polymethine dye. The cyanogen chloride is generated on-line by combining potassium cyanide and chloramine-T. The color is measured at 460 nm (Reference 15.1).
- 2.2 The quality of the analysis is assured through reproducible calibration and testing of the Segmented Flow Analysis (SFA).
- 2.3 A general flow diagram of the SFA system is shown below (see Section 17.0 for a detailed flow diagram).



#### 3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, "Glossary of Definitions and Purposes."

#### 4.0 Interferences

No chemical interferences are known.

#### 5.0 Safety

- 5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.
- 5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.
- 5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.
  - 5.3.1 Acetic Acid, Glacial, CH<sub>3</sub>COOH (FW 60.05)
  - 5.3.2 Chlorimine-T Trihydrate, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>NNaCl•3H<sub>2</sub>0 (FW 281.70)
  - 5.3.3 Nicotine,  $C_{10}H_{14}N_2$  (FW 162.23)
  - 5.3.4 Potassium Cyanide, KCN (FW 65.12)
  - 5.3.5 Sodium Hydroxide, NaOH (FW 40.00)
  - 5.3.6 Sodium Phosphate Dibasic, Na<sub>2</sub>HPO<sub>4</sub> (FW 141.96)
  - 5.3.7 Sulfanilic Acid,  $H_2NC_6H_4SO_3H$  (FW 173.19)
- 5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.
- 5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.
- 5.6 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

#### 6.0 Apparatus, Equipment, and Supplies

- 6.1 Segmented Flow Analysis (SFA) System (OI Analytical Flow Solution IV) consisting of the following:
  - 6.1.1 Model 502 Multichannel Peristaltic Pump
  - 6.1.2 Random Access (RA) Autosampler
  - 6.1.3 Expanded Range (ER) Photometric Detector with 5-mm path length flowcell and 460-nm optical filter
  - 6.1.4 Data Acquisition System (PC or Notebook PC) with WinFLOW<sup>™</sup> software
  - 6.1.5 Nicotine in Tobacco Cartridge (Part #A002714)
- 6.2 Sampling equipment—Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110°–150°C for a minimum of one hour.
- 6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. should all be cleaned, rinsed, and dried per bottle cleaning procedure in Section 6.2.

#### 7.0 Reagents and Calibrants

- 7.1 Raw Materials
  - 7.1.1 Acetic Acid, Glacial, CH<sub>3</sub>COOH (FW 60.05)
  - 7. 1.2 Brij-35<sup>®</sup>, 30% w/v (Part #A21-0110-33)
  - 7.1.3 Chlorimine-T Trihydrate, CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>NNaCl•3H<sub>2</sub>0 (FW 281.70)
  - 7.1.4 Citric Acid, H<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (FW 192.13)
  - 7.1.5 Deionized Water (ASTM Type I or II)
  - 7.1.6 Nicotine,  $C_{10}H_{14}N_2$  (FW 162.23)
  - 7.1.7 Potassium Cyanide, KCN (FW 65.12)
  - 7.1.8 Sodium Hydroxide, NaOH (FW 40.00)
  - 7.1.9 Sodium Phosphate Dibasic, Na<sub>2</sub>HPO<sub>4</sub> (FW 141.96)
  - 7.1.10 Sulfanilic Acid,  $H_2NC_6H_4SO_3H$  (FW 173.19)