

**Summary:** Treat tobacco leaf samples with 0.12 M hydrochloric acid to extract ammonia compounds. Distill at 165 °C and a buffered pH of 9.5. At pH 9.5 all ammonium ions quantitatively convert to ammonia (NH<sub>3</sub>). The NH<sub>3</sub> amount obtained through distillation represents the volatile base. Ammonia reacts with alkaline phenol and hypochlorite to form indophenol blue in an amount that is proportional to the NH<sub>3</sub> concentration. Sodium nitroferrocyanide intensifies the blue color. Measure the absorbance at 640 nm.

**Interferences:** Filter turbid samples prior to analysis. Eliminate precipitation in the distillation tubing by adding ethylenediaminetetraacetic acid (EDTA). Samples with background absorbance at the analytical wavelength may interfere.

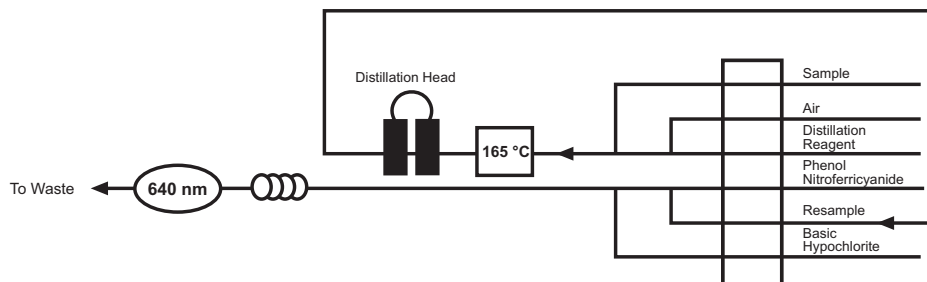
**Performance Specifications:**

Range	0.02–0.40% volatile base as NH <sub>3</sub>
Throughput	25 samples/hour
Precision at 0.04%	<3.29% RSD
0.2%	<3.75% RSD
0.4%	<2.62% RSD
Method Detection Limit (MDL)	0.01%

**Chemicals:**

Ammonium sulfate, (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (FW 132.14)	Phenol, solid or liquified, 88%, C <sub>6</sub> H <sub>5</sub> OH (FW 94.11)
Brij <sup>®</sup> -35, 30% w/v (PN A21-0110-33)	Sodium hydroxide, NaOH (FW 40.00)
EDTA, C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>8</sub> •2H <sub>2</sub> O (FW 372.24)	Sodium hypochlorite, 5.25% available chlorine (household bleach), NaOCl (FW 74.44)
Hydrochloric acid, concentrated, HCl (FW 36.46)	Sodium nitroferrocyanide dihydrate, Na <sub>2</sub> Fe(CN) <sub>5</sub> NO•2H <sub>2</sub> O (FW 297.95)
Kleenflow <sup>™</sup> Acidic (PN A001251)	Sodium tetraborate decahydrate, Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> •10H <sub>2</sub> O (FW 381.37)
Kleenflow Basic (PN A001252)	

**Basic Flow Diagram:**



**Selected References:** Nitrogen, Ammonia. *Methods for Chemical Analysis of Water and Wastewater*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; Method 350.1.

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