

# Determination of Methyl Nitrite in Cigarette Smoke

C. H. Sloan and B. J. Sublett

Research Laboratories, Tennessee Eastman Company, Division of Eastman Kodak Company, Kingsport, Tennessee, U.S.A.

## Introduction

Methyl nitrite has been identified in cigarette smoke by Phillippe and Hackney (3). They collected smoke from several cigarettes, fractionated the smoke components, and, by using infrared spectroscopy, identified and estimated the amount of methyl nitrite in an isolated fraction.

This paper describes a relatively simple, rapid technique which utilizes gas-liquid chromatography and spectrophotometry to isolate and measure methyl nitrite from the smoke of one cigarette. The spectrophotometric method involves the use of the well-known Griess reagent for determining nitrite as reported by Saltzman (4).

## Materials and Methods

### Apparatus.

1. Smoking machine—Cigarettes were smoked on an automatic smoking machine which was a modified version of the one described by Mumpower, Kiefer, and Touey (2). Figure 1 is a diagram of the machine.
2. Gas chromatographic equipment—Figure 2 is a diagram of the gas chromatographic equipment and the sample inlet system. The apparatus consisted of a gas sampling valve, a gas collection trap, a gas-liquid chromatographic column, and a detector. The gas collection trap was a 12-in. length of coiled  $\frac{1}{4}$ -in. stainless steel tubing attached from the ends of the trap to the gas sampling valves with two 6-in. lengths of  $\frac{1}{8}$ -in.-o.d. Teflon tubing.

The gas-liquid chromatographic column was a 3/16 in. copper tube 10 ft in length which was packed with 60-80 mesh Chromosorb W uniformly impregnated with 20 percent by weight 3,3'-oxydipropionitrile. The column was operated at room temperature.

A thermal conductivity cell (Gow-Mac, W-2 tungsten filaments) operating at room temperature (150 ma on the bridge) was used as the detector. Helium was used as the carrier gas with a flow rate through the column of 40 ml per min.

3. Spectrophotometer—A Beckman Model B spectrophotometer with two 10-mm cuvettes was used.
4. Cambridge filter assembly—A Cambridge filter holder with 45-mm Cambridge filter pads (CM-113A) was used.

### Reagents.

1. Griess reagent—Five grams of sulfanilic acid was placed in a one-l volumetric flask. Six hundred ml of water and 140 ml of glacial acetic acid were added, and the mixture was stirred until the sulfanilic acid dissolved. N-1-Naphthylethylenediamine dihydrochloride (0.2 g) was placed in a 100-ml volumetric flask and was diluted to a volume of 100 ml with distilled water. Twenty ml of the N-1-naphthylethylenediamine dihydrochloride solution was added to the

sulfanilic acid solution and it was diluted to a volume of one l with distilled water. The reagent solution should be stored in an amber bottle.

2. Methyl nitrite—a gas train was assembled that consisted respectively of a finger-type reactor containing five ml of methanol; a finger-type trap containing a solution of  $\text{KMnO}_4$ ,  $\text{NaNO}_3$ , and  $\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$ ; a drying tube filled with indicating Drierite and anhydrous  $\text{MgSO}_4$ ; and an empty finger-type trap cooled to approximately  $-70^\circ\text{C}$  with dry ice and isopropyl alcohol. A mixture of nitrogen dioxide and air was passed through the system and the methyl nitrite formed was condensed in the cold trap. After approximately two ml of condensate had formed in the trap, the trap was removed from the gas train, cooled with liquid nitrogen, and the volatile gases were removed under a reduced pressure of 0.1 mm. The methyl nitrite was transferred to an evacuated stainless steel cylinder by attaching the cylinder to the cold trap, immersing the cylinder in liquid nitrogen, and allowing the methyl nitrite to distill from the trap into the cylinder. This operation further purified the sample since methyl nitrate and other less volatile materials which may have been formed during the reaction were not distilled from the cold trap. A sample

of the methyl nitrite was chromatographed on the column previously described. The absence of extraneous peaks indicated the sample was relatively pure.

**Cigarettes.** All the cigarettes used in these experiments were 85 mm in length, conditioned to 12% moisture content, and each group weighed  $\pm 20$  mg of their average weight. All the cigarettes were smoked to a 30 mm butt length.

**Calibration Curve for Methyl Nitrite.** A benzene solution containing  $3.5 \pm 0.1$  mole percent methyl nitrite was prepared by passing methyl nitrite from a storage cylinder into benzene. The concentration of methyl nitrite in the benzene solution was determined with a Varian A-60 NMR spectrometer.

The concentration of methyl nitrite in micrograms per milliliter was calculated from the equation:

$$\text{Conc of methyl nitrite, } \mu\text{g/ml} = \frac{\text{M.W. CH}_3\text{ONO}}{\text{M.W. Benzene}} \times \frac{\text{Moles CH}_3\text{ONO}}{\text{100 - moles CH}_3\text{ONO}} \times \frac{\text{Density of Benzene} \times 10^6}{\text{One ml of the benzene solution containing 25,300 } \mu\text{g of methyl nitrite was pipetted into a volumetric flask and diluted to 100 ml with benzene. Six duplicate aliquots of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ml of this solution were pipetted into twelve 250 ml volumetric flasks, each containing 50 ml of Griess reagent. The solutions were allowed to stand 15 min until maximum color developed and then were diluted to 250 ml with distilled water. Absorbance was read on the spectrophotometer at 550 m}\mu\text{ with Griess reagent and water as the blank. The calibration curve shown in Figure 3 was prepared by plot-$$

ting absorbance versus  $\mu\text{g}$  of methyl nitrite.

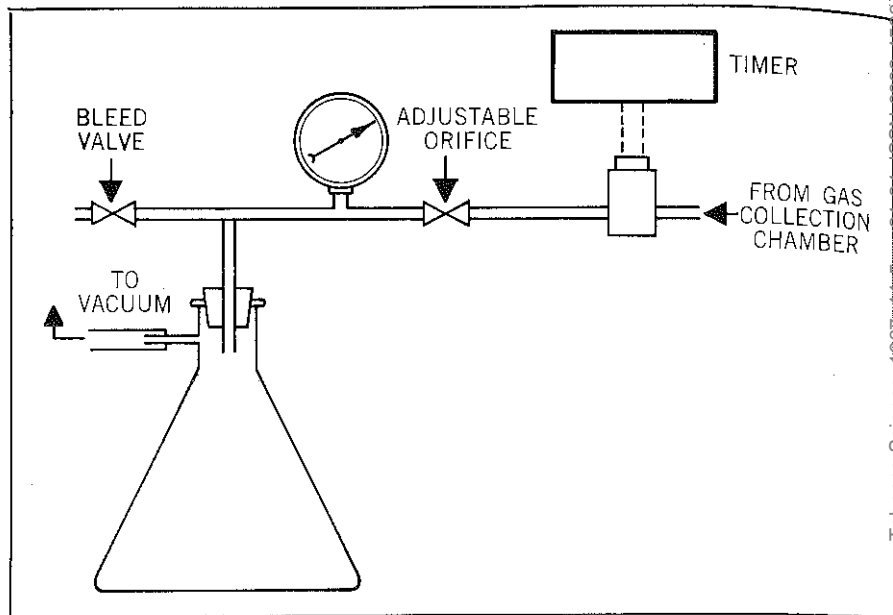


Figure 1. Smoking apparatus.

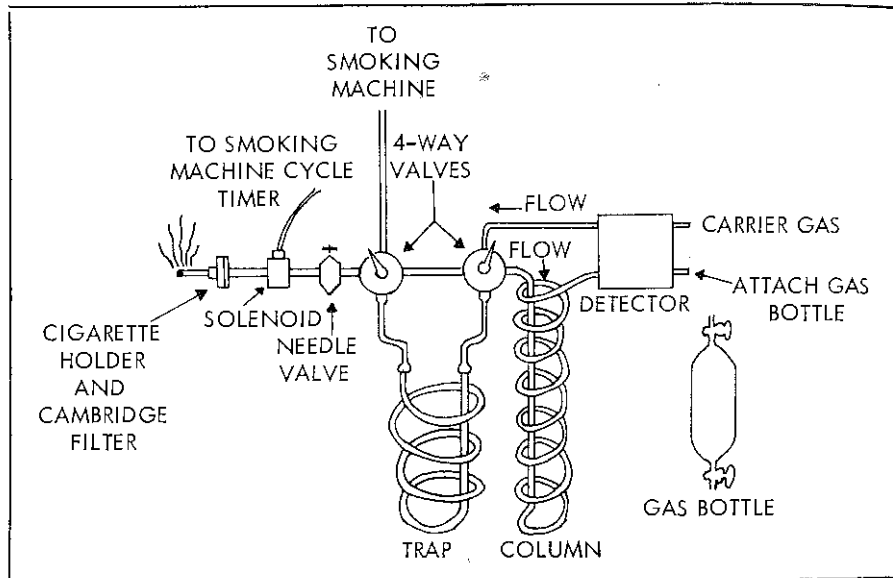


Figure 2. Multiple-puff sampling apparatus.

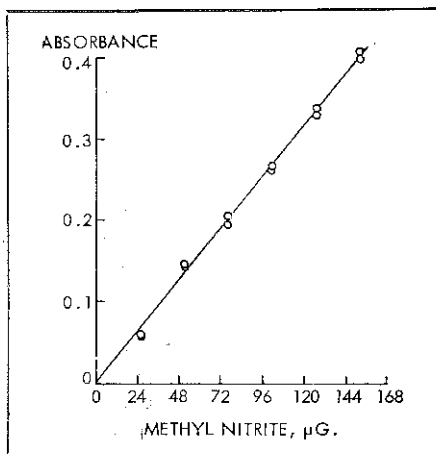


Figure 3. Calibration curve for methyl nitrite.

ting absorbance versus  $\mu\text{g}$  of methyl nitrite.

**Calibration Curve for Nitrite.** A calibration curve with a range of 1 to 120  $\mu\text{g}$  of nitrite was prepared from spectrophotometric measurements of color produced by the reaction of aliquots of sodium nitrite solution with Griess reagent. The aliquots of sodium nitrite solution were combined with 50 ml of Griess reagent and allowed to react 15 min. The samples were diluted to a volume of 250 ml with distilled water. The optical density was read from

the Beckman spectrophotometer at a wavelength of 550 m $\mu$ .

#### Procedure

The Cambridge filter holder was attached to the gas sampling valve as shown in Figure 2. The gas collection trap (stainless steel tube) was immersed in a dewar flask filled with liquid nitrogen and the gas valves on the smoking machine and gas collection trap were adjusted to take a 35 ml puff of two-sec duration at the rate of one puff

per min. The solenoid valve attached to the gas collection trap was synchronized to open and close with the solenoid on the smoking machine. This prevented the vacuum created by cooling the gas collection trap with liquid nitrogen from applying a continuous draw to the lighted cigarette and limited the puff to a 35 ml volume of two-second duration. The cigarettes were placed in the Cambridge filter holder and smoked to a 30-mm butt length. Cigarettes were then removed and five puffs of air taken through the filter assembly to sweep residual vapors into the gas collection trap.

The gas valves were adjusted to route the carrier gas through the trap and chromatographic column. The dewar flask containing liquid nitrogen was immediately replaced with a dewar flask containing dry ice and isopropyl alcohol which warmed the trap to approximately  $-70^{\circ}\text{C}$ .

The gas collection trap remained immersed at  $-70^{\circ}\text{C}$  until the carrier gas had carried the volatile materials out of the trap and through the chromatographic system. This operation required approximately 20 min. After the system had equilibrated, the  $-70^{\circ}\text{C}$  cold trap was replaced by a dewar flask filled with water ( $25^{\circ}\text{C}$ ). Two peaks with retention times of 1 min 48 sec and 3 min represented the impure fractions of nitric oxide and methyl nitrite, respectively, which were eluted from the column. Just before the methyl nitrite was eluted from the column, a one-l evacuated gas bottle containing 50 ml of Griess reagent was placed on the detector exhaust. The elution of methyl nitrite into the gas bottle was followed by observing the recorder pen. After the methyl nitrite had been eluted from the column into the gas bottle, the gas bottle, which was still under re-

duced pressure, was vented to the atmosphere to fill the bottle with air. The gas bottle was then shaken for 15 min to allow the characteristic red-violet color to develop to maximum intensity. After the contents of the bottle were emptied into a 250-ml volumetric flask, the bottle was washed twice with distilled water and the washings were added to the flask. The solution was diluted to a total volume of 250 ml with distilled water. The absorbance was determined spectrophotometrically against a blank of Griess reagent and water at a wavelength of  $550\text{ m}\mu$ .

### Results and Discussion

The chromatographic column used for the analysis of methyl nitrite in cigarette smoke was a modification of the column described by Harrison and Stevenson (1). This column effectively separates alkyl nitrites; however, when it is used for the separation of alkyl nitrites in cigarette smoke, the nitrites are not completely separated from other components in the smoke. Methyl nitrite is eluted from the column with several other compounds, the main portion of which is methyl chloride. Although methyl nitrite is not separated from cigarette smoke by this system in a pure state, it is separated from other alkyl nitrites and species such as nitric oxide which after oxidation to nitrite will develop a characteristic color with Griess reagent. A typical chromatogram of the impure peak representing methyl nitrite is shown in Figure 4. The identification of methyl nitrite was made by trapping the components as they were eluted from the chromatographic column and by analyzing the trapped components by mass spectrometry. The retention time of a chromatographed known sample of methyl nitrite conforms to that of a sample trapped from to-

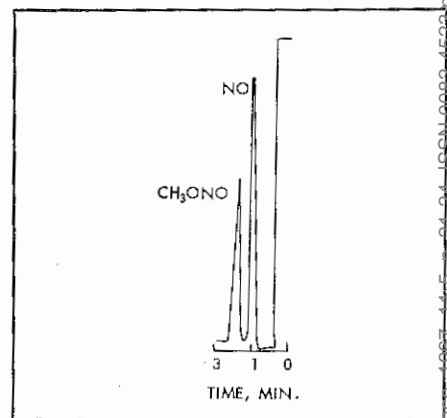


Figure 4. Observed chromatogram of methyl nitrite.

bacco smoke. Due to the impure state of the eluate containing methyl nitrite, quantitative measurements cannot be made by peak height or peak area measurements. Therefore, the chromatographic method in the procedure is used only as a means of separating methyl nitrite from other species that produce color with Griess reagent, and not as an analytical tool. Figure 5 shows two calibration curves prepared from methyl nitrite and sodium nitrite as described previously. The points on the curve were plotted as micrograms of nitrite versus absorbance. Considering the experimental errors involved in the preparation of the methyl nitrite curve, it appears that sodium nitrite could be used to calibrate for methyl nitrite with reasonable accuracy for samples containing small quantities of methyl nitrite.

To determine the stability of methyl nitrite under the conditions used for analysis, an experiment was designed which consisted of smoking and analyzing the smoke samples of 1, 2, 3 and 4 average

Table 1. Methyl nitrite in cigarette smoke

Tobacco Type	Weight of Cigarette, g	Number of Cigarettes Smoked	$\text{NO}_2^-$ on Tobacco, %	Methyl Nitrite per Cigarette, $\mu\text{g}^a$
Bright	$1.2025 \pm 20\text{ mg}$	5	0.035	25
Burley	$0.9932 \pm 20\text{ mg}$	5	1.17	94
Commercial Blend	$1.1650 \pm 20\text{ mg}$	5	0.57	67
Turkish	$1.3932 \pm 20\text{ mg}$	5	0.0815	19
Burley	$1.1960 \pm 20\text{ mg}$	5	1.45	48

<sup>a</sup> Average of five determinations.

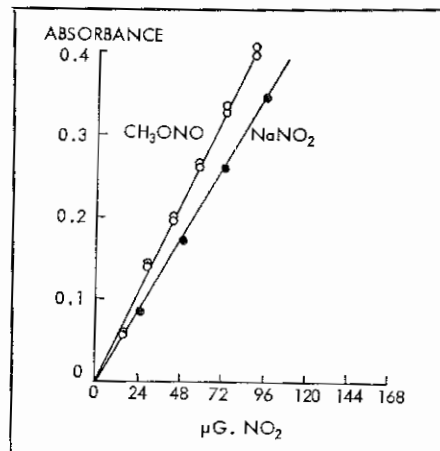


Figure 5. Calibration curves made from known samples of methyl nitrite and sodium nitrite.

**Table 2. Relative standard deviation for five replicate analyses of methyl nitrite in cigarette smoke**

	Methyl Nitrite per Cigarette, $\mu\text{g}$		
	Bright	Burley	Com. Blend
	20	88	61
	25	94	60
	28	92	58
	23	98	57
	27	98	65
Mean	24.6	94.0	60.2
Standard deviation	$\pm 3.1$	$\pm 4.24$	$\pm 3.1$
% Relative Standard deviation	12.6	4.51	5.15

**Table 3. Methyl nitrite content of methanol-treated cigarettes**

Cigarettes	Methanol Added	Methyl Nitrite per Cigarette, $\mu\text{g}$
8% Alkali Nitrate	50 $\mu\text{l} \cong 39.8 \text{ mg}$	250
8% Alkali Nitrate	50 $\mu\text{l} \cong 39.8 \text{ mg}$	235
		Mean 242.5
8% Alkali Nitrate	None	93
8% Alkali Nitrate	None	94
		Mean 93.5
0.6% Nitrate <sup>a</sup>	50 $\mu\text{l} \cong 39.8 \text{ mg}$	98
0.6% Nitrate <sup>a</sup>	50 $\mu\text{l} \cong 39.8 \text{ mg}$	104
		Mean 101
0.6% Nitrate <sup>a</sup>	None	56
0.6% Nitrate <sup>a</sup>	None	58
		Mean 57.5

<sup>a</sup> Commercial blend.

weight cigarettes, respectively. All the cigarettes were smoked to a 30 mm butt length. These data are shown in Figure 6 as micrograms of methyl nitrite versus the number of cigarettes smoked. These data indicate that methyl nitrite is stable under the conditions of analysis and is not formed in the cold trap. The data also indicate that the amount of methyl nitrite found in the smoke is dependent upon the amount of tobacco smoked.

Methyl nitrite was determined in the smoke from five groups of cigarettes which are shown in Table 1. The nitrate contents and average weights of the cigarettes are given to emphasize the differences among the groups of cigarettes. For example, the two groups of Burley cigarettes shown are from different sources, and were manufactured on different equipment. Both of these parameters could affect the uniformity and burning characteristics of the cigarettes.

Other parameters that might affect the precision of the method are: smoking of the cigarettes in an atmosphere with a variable humidity, the reproducibility of the elution of methyl nitrite from the chromatographic column, and variation in pressure drop (resistance to air flow through the cigarettes).

All of the cigarettes in each group were of average weight, from the same tobacco, and conditioned in the same manner. However, the uniformity of the cigarettes and burning characteristics among the groups of cigarettes varied considerably. Since these two parameters affect the reproducibility of the method, the percent relative standard deviation was calculated for each group of cigarettes smoked. The relative standard deviations for three of the groups are shown in Table 2. The percent relative standard deviation was calculated from five individual determinations which consisted of smoking one cigarette per determination.

In order to study the source of methyl nitrite in cigarette smoke, two types of cigarettes were treated with methanol and smoked, and the methyl nitrite was determined. The methyl nitrite content of the smoke from the treated cigarettes was compared with the methyl nitrite content of the smoke from the respective control cigarettes which had not been treated with methanol. These data are given in Table 3. The cigarettes used were a commercial blend and a cigarette made from tobacco containing eight percent by weight alkali nitrate.

In both types of cigarettes to which methanol was added, the

methyl nitrite content of the smoke increased. These data indicate that methyl nitrite is formed as a vapor phase reaction from methanol and oxides of nitrogen during the smoking of a cigarette. The data also indicate that the amount of methyl nitrite in cigarette smoke is dependent upon the amount of methanol on the tobacco or the methanol formed as a combustion product of tobacco.

### Summary

A method has been developed for the determination of methyl nitrite from the smoke of one cigarette. The method is useful for comparing the methyl nitrite content of the smoke from different types of cigarettes and for evaluating filters which might remove methyl nitrite from cigarette smoke. The method could also be used for the determination of alkyl nitrites in complex mixtures other than cigarette smoke on which direct analysis cannot be made.

### Literature Cited

- Harrison, R. M., and F. J. Stevenson, Gas chromatography analysis of alkyl nitrites, *J. Gas Chromatogr.*, 240-241. July, 1965.
- Mumpower, R. C., J. E. Kiefer, and G. P. Touey, Effect of the duration of a constant-volume puff on the efficiency of cigarette filters, *Tobacco Sci.*, 5: 31-35. 1961.
- Phillips, R. J., and E. J. Hackney, The presence of nitrous oxide and methyl nitrite in cigarette smoke and tobacco pyrolysis gases, *Tobacco Sci.*, 3: 139-143. 1959.
- Saltzman, B. E., Colorimetric microdetermination of nitrogen dioxide in the atmosphere, *Anal. Chem.*, 26: 1949-1954. 1954.

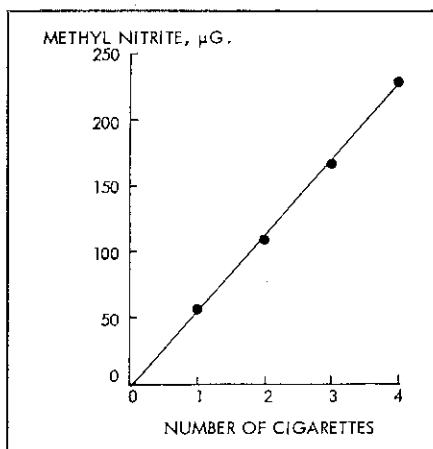


Figure 6. Correlation of methyl nitrite with amount of tobacco smoked.