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Note

Rapid method for the analysis of volatile N-nitrosamines in cigarette smoke by glass capillary chromatography

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It has been well established that many N-nitrosamines are highly carcinogenic[1–3]. Since Druckrey and Preussman[4] first made the suggestion in 1962 that favorable conditions exist in cigarette smoke for the production of N-nitrosamines, there has been considerable interest in the examination of cigarette tar for the presence of N-nitrosamine compounds[5,6]. These studies have required elaborate enrichment procedures for the concentration, detection and quantitative analyses of cigarette smoke nitrosamines. Brunnemann *et al.*[7] reported a fairly simple extraction procedure followed by the use of the highly sensitive and specific thermal energy analyzer (TEA), developed by Fine *et al.*[8]. However, many laboratories, including our own, are not equipped with the expensive TEA. We have, therefore, developed an alternative method for the analysis of volatile N-nitrosamines employing glass capillary gas chromatography (GC) and a nitrogen–phosphorus thermionic detector (NPD). This method has enabled us to monitor dimethylnitrosamine (DMNA), diethylnitrosamine (DENA) and nitrosopyrollidine (NPYR) in the smoke from various types of cigarettes.

EXPERIMENTAL*

Materials

Cigarette smoke condensate (CSC) was collected on a 30-port Borgwaldt smoking machine. Standard smoking conditions were used: 1 puff/min, puff duration of 2 sec, puff volume of 35 ml and butt length of 23 mm. The smoke from 60 cigarettes was collected in 200 ml of a citric acid–sodium phosphate buffer solution (pH 4.5) to which 20 nm of ascorbic acid had been added.

Standard nitrosamines were obtained from Eastman Chemical and used without further purification.

Extraction procedure

The extraction procedure was similar to that reported by Hoffmann *et al.*[9]. The

* Reference to a company or product name does not imply approval or recommendation by the United States Department of Agriculture.

buffer solution was extracted three times with 100 ml of dichloromethane. The combined organic extracts were dried over sodium sulfate, concentrated to 5 ml and chromatographed on 30 g of basic alumina (activity II to III) contained in a 200 × 20 mm glass column. The column was eluted with 250 ml of dichloromethane and 250 ml of dichloromethane–acetone (4:1, v/v). The dichloromethane eluate was concentrated on a rotary evaporator to 1 ml for GC analysis.

Glass capillary GC analysis

The samples were analyzed with a Hewlett-Packard Model 5710A gas chromatograph equipped with a NP detector. The standard 5710A instrument was modified for glass capillary GC analyses as previously described[10]. The nitrosamines were analyzed in the split mode (100:1) with a 1-μl sample on a 10 m × 0.25 mm I.D. wall-coated open tubular (WCOT) column of Superox™-4, a 4,000,000 mol. wt. polyethylene glycol. The column was prepared according to Arrendale and coworkers[11,12]. The NPD was operated under hydrogen and air flow conditions as recommended by the manufacturer. The injection-port temperature was 200°C, the detector temperature was 250°C and the temperature program was 70°C for 8 min. then 2°C/min to 250°C.

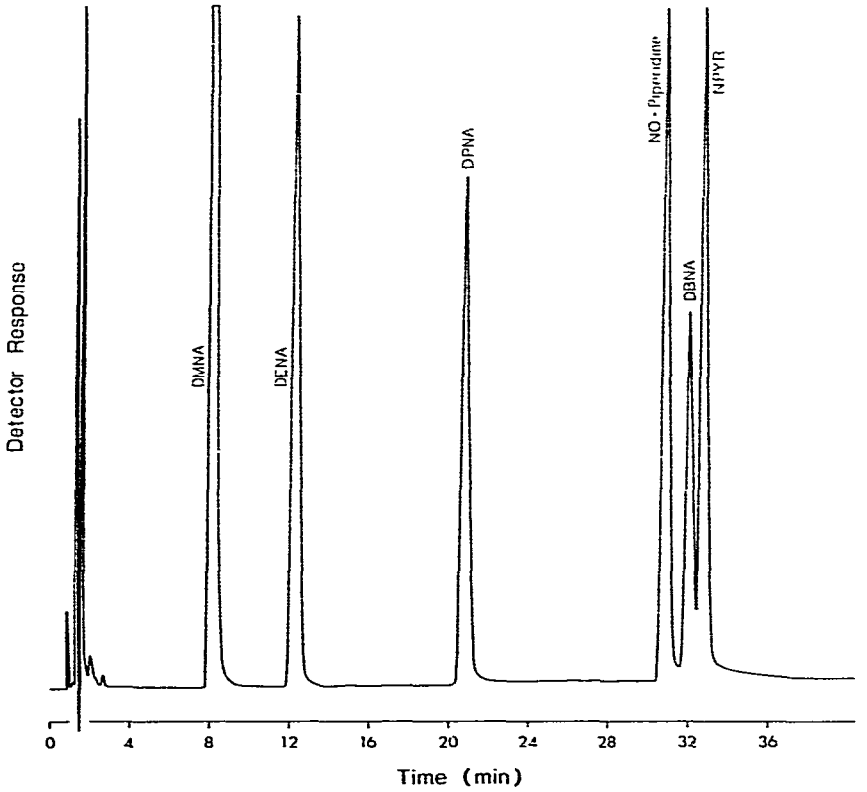

Fig. 1. Glass capillary gas chromatogram of standard nitrosamines on a Superox™-4 WCOT column. DMNA = Dimethylnitrosamine. DENA = diethylnitrosamine. DPNA = dipropylnitrosamine. DBNA = dibutylnitrosamine. NPYR = nitrosopyrollidine.

RESULTS AND DISCUSSION

Brunnemann and Hoffmann[13] used GC on a packed column of 10% Carbowax 20M to separate and quantitate the volatile CSC N-nitrosamines. We felt that because of their separation efficiencies WCOT glass capillary columns could be utilized to resolve better the nitrosamines and to shorten analysis time. The chromatogram of the standard N-nitrosamine mixture on a Superox™-4 WCOT column is shown in Fig. 1.

Since CSC is such a complex mixture almost any separation will still yield an impure and heterogeneous mixture. Therefore, we felt that a NPD would eliminate any interferences of non-nitrogen containing compounds in the quantitation of the volatile N-nitrosamines. Also, the increased sensitivity of the NP detector over the flame-ionization detector would be helpful, as the nitrosamines are present in the ng/cigarette range. As can be seen even with the NPD, the resulting CSC chromatograms were quite complex (Fig. 2). However, the major volatile N-nitrosamines, DMNA, DENA and NPYR, can be detected.

Our primary aim in developing a rapid method for the analysis of volatile nitrosamines was to evaluate the smoke from various tobacco samples for content of these carcinogenic materials. As a comparison to previous methods, quantitative data

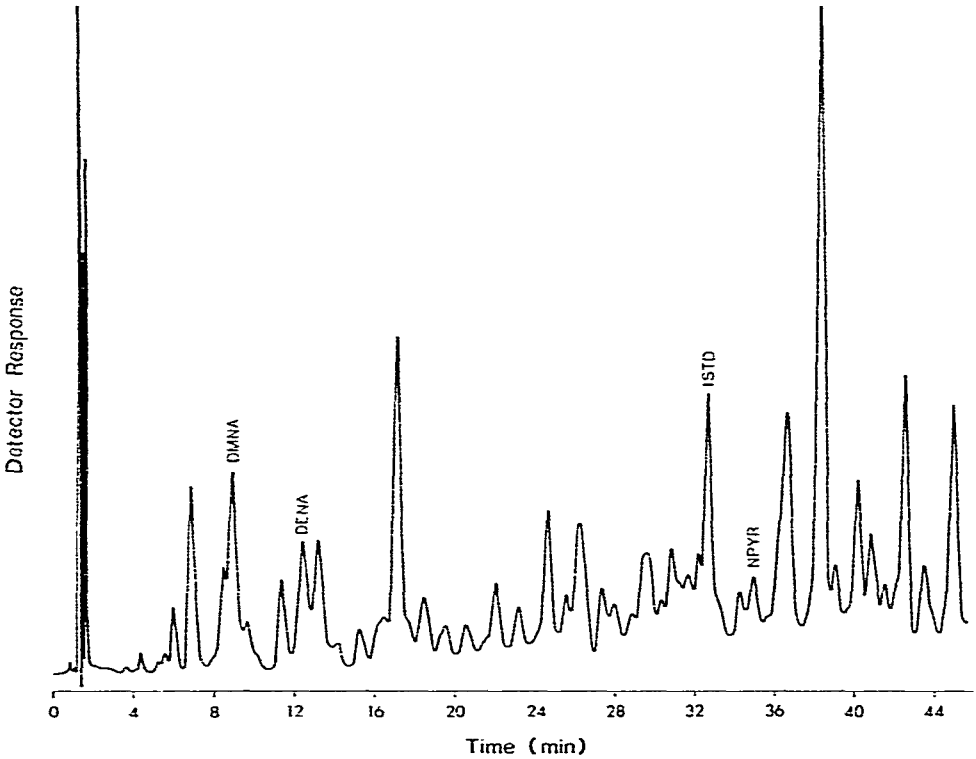

Fig. 2. Glass capillary gas chromatogram of a fraction derived from the CSC of Kentucky 2R1 cigarettes on a Superox™-4 WCOT column. DMNA = Dimethylnitrosamine, DENA = diethylnitrosamine, ISTD = internal standard (nitrosopiperidine), NPYR = nitrosopyrollidine.

from Brunnemann and Hoffmann[13] and our method are compared in Table I for three different types of cigarettes. Except for the filter cigarette our values are higher than those reported by Brunnemann and Hoffmann. These differences may be due to the manner in which the smoke was collected or to some interference from other nitrogen-containing substances in the CSC fraction that were not detected by the TEA. However, we feel this method provides us with an adequate tool for determining differences in volatile nitrosamine contents of various cigarettes. This procedure permits a laboratory not equipped with an expensive TEA system to monitor the contents of the highly carcinogenic volatile nitrosamines in CSC. Also, this method should have equal or better applicability to the analyses of volatile nitrosamines in simpler environmental or food samples.

TABLE I

COMPARISON OF DATA FROM TEA* AND NPD METHODS

	Nitrosamine contents (μg/cigarette)					
	TEA			*NPD*		
	DMNA	*DENA*	*NPYR*	*DMNA*	*DENA*	*NPYR*
Kentucky 1R1	9.0	2.0	6.6	17.0	5.8	1.5
Commercial non-filter	13.0	1.5	11.0	18.2	17.1	9.9
Commercial filter	5.7	1.3	5.1	0.75	9.9	0.08

* Brunnemann and Hoffmann[13].

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REFERENCES

1 H. Druckrey, R. Preussman, S. Ivankovic and D. Schmu, *Z. Krebsforsch.*, 69 (1967) 103.
2 B. Toth and P. Shubik, *Cancer Res.*, 27 (1967) 43.
3 D. P. Griswold, Jr., A. E. Casey, E. K. Weisburger, J. H. Weisburger and F. M. Schnabel, Jr., *Cancer Res.*, (1966) 619.
4 H. Druckrey and R. Preussman, *Naturwissenschaften*, 49 (1962) 498.
5 W. J. Serfontein and P. Hunter, *Cancer Res.*, 26 (1966) 575.
6 C. Ruhl, J. D. Adams and D. Hoffman, *J. Anal. Tox.*, 4 (1980) 255.
7 K. D. Brunnemann, L. Yu and D. Hoffmann, *Cancer Res.*, 37 (1977) 3218.
8 D. H. Fine, F. Rufeh, D. Lieb and B. P. Rounbehler, *Anal. Chem.* 47 (1975) 1188.
9 D. Hoffmann, J. D. Adams, K. D. Brunnemann and S. S. Hecht, *Cancer Res.*, 39 (1979) 2505.
10 R. F. Severson, R. F. Arrendale and O. T. Chortyk, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 11.
11 R. F. Arrendale, L. B. Smith and L. B. Rogers, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 208 (1981) 209.
12 R. F. Arrendale, R. F. Severson and O. T. Chortyk, *J. Chromatogr.*, 208 (1981) 209.
13 K. D. Brunnemann and D. Hoffmann, *IARC Scientific Publications*, 19 (1978) 343.