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Note

Chemical studies on tobacco smoke

LXXV. Rapid method for the analysis of tobacco-specific N-nitrosamines by gas–liquid chromatography with a thermal energy analyser*

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The occurrence of N′-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosoanatabine (NAT) in tobacco and tobacco smoke has been ascertained during recent years[1,2]. Together these carcinogenic nitrosamines range from 0.1 to 100 ppm in processed tobacco and from 0.4 to 9.0 μg per cigarette in mainstream smoke[1]. In earlier studies, lengthy enrichment procedures, followed by gas and thin-layer chromatographic techniques led to the identification of NNN in tobacco smoke condensate[3]. Later, high-performance liquid chromatography (HPLC) with UV detection was successfully used for the quantitative analysis of NNN and NNK[4,5]. Munson and Abdine[6] refined the gas chromatographic (GC) procedure by using a mass spectrometer as detector, analyzing quantitatively for NNN in various tobaccos. A more recently developed method required only a relatively simple enrichment procedure and HPLC analysis with the thermal energy analyzer (TEA) as a detector[1]. This method has recently been applied by Andersen *et al.*[7] for the analysis of NNN in tobacco samples. Though not as tedious as the earlier methods, this procedure was still rather cumbersome. Chamberlain *et al.*[8] recently reported on the analysis of NNN, NAT and NNK in tobacco and cigarette smoke using capillary GC with a nitrogen–phosphorus detector (NPD); however, they encountered interferences because of the multitude of nitrogenous components present in tobacco and cigarette smoke responding also to the NPD.

The objective of this study was, therefore, to develop a rapid method for the analysis of NNN, NNK, NAT and the newly identified carcinogenic nitrosamine N′-nitrosoanabasine (NAB). An optimized GC–TEA system, similar to that for the analysis of N-nitrosodiethanolamine, was devised[9]. The tobacco-specific N-nitrosamines (TSNA) were determined and the GC–TEA data were compared with those obtained by HPLC–TEA.

* Dedicated to the founder of the American Health Foundation, Dr. Ernst L. Wynder, on the occasion of the 10th anniversary of the Naylor Dana Institute for Disease Prevention.

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MATERIALS AND METHODS

Apparatus

For the GC–TEA analyses, a Hewlett-Packard Model 700 gas chromatograph was interfaced with a modified Model 543 TEA as previously described[9]. A 4.5 m × 2 mm I.D. glass column packed with 10% UCW-982 on Gas-Chrom Q (80–100 mesh) was used. For the HPLC–TEA analyses, a Waters Assoc. Model M-45 solvent-delivery system was utilized with a Rheodyne Model 7105 syringe-loading sample injector; this system was interfaced with a Model 502/LC TEA. The separation was achieved on a pair of LiChrosorb Si-60 columns (Merck, Darmstadt, G.F.R.). Mass spectrometry (MS) was carried out using a Hewlett-Packard Model 5892A GC–MS instrument.

Reagents

All N-nitroso reference compounds were prepared in our laboratory according to previously published methods[1,10–12]. All solvents were purchased from Fisher Scientific (Pittsburgh, PA, U.S.A.) and were of spectrograde quality. All other reagents were purchased from local distributors.

Cigarettes and tobacco

The cigarettes and tobacco used were purchased in Westchester County, NY, U.S.A., in 1981.

Sample preparation and analysis

The TSNA in cigarette smoke or tobacco were enriched according to previously published methods[1]. This involved extractions of trapped cigarette smoke or of tobacco with citrate buffer (pH 4.5) containing 20 m*M* ascorbic acid and addition of [^{14}C]NNN as an internal standard. The aqueous extract was partitioned with dichloromethane, concentrated and chromatographed on 90 g of basic alumina (Woelm, activity II–III). The TSNA fractions were pooled, concentrated and analyzed by GC–TEA or HPLC–TEA. For the HPLC–TEA analysis, previously described methods were used[1].

For the GC–TEA method, a 4.5 m × 2 mm I.D. glass column was washed successively with acetone, hexane, acetone, methanol and acetone. The column was thoroughly dried for 30 min and then filled with a 10% solution of dimethyldichlorosilane in toluene. After this, the column was rinsed thoroughly with methanol, dried and packed with 10% UCW-982 on Gas-Chrom Q (80–100 mesh). Argon was allowed to flow for 5 min, was then discontinued and the column was heated to 290°C. After 30 min the column was cooled to room temperature and the gas flow was reestablished. Then the column was heated at 260°C for 30 min. This conditioning procedure was found to be necessary to obtain a separation between NAT and NAB. The GC conditions were carrier gas (argon) flow-rate 37 ml/min, injector temperature 200°C and oven temperature 190°C.

For the MS identification of NAB in tobacco, 500 g (dry weight) of fine-cut chewing tobacco were extracted with 2 l of citrate buffer (pH 4.5) containing 7 g of ascorbic acid as an inhibitor. After 24 h the extract was filtered and freeze-dried. The residue was dissolved in water and extracted with dichloromethane. The organic

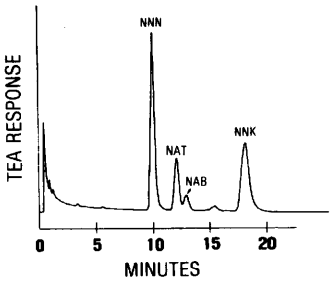

Fig. 1. GC–TEA trace of TSNA in cigarette smoke.

phase was dried, concentrated and chromatographed on silica gel. TEA monitoring was used to determine the NAB-containing fractions which were combined, concentrated and injected into a normal-phase HPLC system. The collected NAB band was re-chromatographed by reversed-phase HPLC and then extracted, concentrated and analyzed by GC–MS.

For the identification of NAB in cigarette smoke, the smoke from 500 non-filter cigarettes was trapped in a buffered ascorbic acid solution, which was then extracted with dichloromethane; further purification was achieved by the chromatographic procedures described above.

For GC–MS a 25 m × 0.25 mm I.D. Carbowax 20M fused-silica capillary column (film thickness 0.25 μm) was utilized. The system was used under splitless conditions with a column pressure of 10 p.s.i. The oven temperature was held at 65°C for 2 min and then increased at 8°C/min to 200°C. Under these conditions NAB eluted at 23.8 min.

RESULTS AND DISCUSSION

A typical GC–TEA trace of the TSNA fraction from cigarette smoke is shown

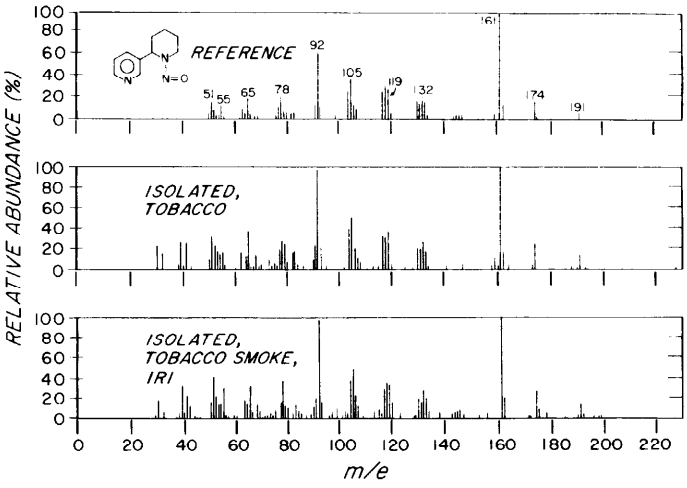

Fig. 2. Mass spectra of synthetic NAB and NAB isolated from snuff tobacco and tobacco smoke (1R1 Kentucky Reference cigarette).

TABLE I

COMPARISON OF GC–TEA AND HPLC RESULTS

All values are the means of three analyses.

Sample	*Compound*	*Units*	*HPLC–TEA*	*GC–TEA*
Tobacco*	NNN	μg/g	46.0 ± 9%	45.6 ± 7%
	NNK		7.19 ± 9%	7.38 ± 8%
	NAT		47.8 ± 16%	47.7 ± 8%
	NAB**		–	1.8 ± 12%
Cigarette*** mainstream smoke	NNN	ng per cigarette	935 ± 7%	954 ± 6%
	NNK		780 ± 20%	767 ± 5%
	NAT		979 ± 20%	990 ± 7%
	NAB**		–	120 ± 10%

* Fine-cut chewing tobacco.
** NAB could not be quantitated by HPLC–TEA.
*** U.S.A. 85-mm cigarette without filter tip.

in Fig. 1. While NAT and NAB co-eluted in the HPLC TEA method, GC TEA resolved a new TSNA, which was identified as NAB. Fig. 2 shows mass spectra for NAB isolated from tobacco and tobacco smoke compared with that of authentic reference NAB.

Table I compares the data obtained with the two different methods. The values are the arithmetic mean of three analyses. It is apparent that both methods are in good agreement; however, analysis by GC–TEA is more precise and offers several other advantages over the HPLC–TEA method. It allows for the separation of NAT and the newly identified NAB, which is not possible by HPLC, and it is more economical and faster than the HPLC method.

A direct comparison of our data with those obtained by other groups is not possible because of the different detection systems used; also, none of the samples were identifiable to us to allow comparison.

Previous efforts to analyze the TSNA by GC–TEA failed because of sample degradation caused by the standard TEA interface. These difficulties are overcome by using an all-glass system with on-column injection and direct interface of the GC column to the ceramic pyrolysis tube of the TEA.

In summary, a method that allows the rapid analysis of four tobacco-specific N-nitrosamines representing the most abundant carcinogens in tobacco and tobacco smoke has been developed. This method could also be applied to other N-nitroso compounds that were previously not amenable to GC–TEA analysis.

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