MULTIDIMENSIONAL GAS CHROMATOGRAPHIC DETERMINATION OF COTININE AS A MARKER COMPOUND FOR PARTICULATE-PHASE ENVIRONMENTAL TOBACCO SMOKE*

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SUMMARY

Multidimensional gas chromatographic analysis of air particles for the tobacco alkaloid cotinine is described. The analytical procedure requires little sample preparation. Unambiguous identification of cotinine and nicotine in cigarette smoke and indoor air samples was achieved by precise, reproducible retention times observed with two parallel analytical columns of different polarities and a nitrogen-specific detector. Further investigation of smoking and environmental variables is needed to validate the use of cotinine as a marker compound for environmental tobacco smoke particulate matter.

INTRODUCTION

Much concern has been expressed regarding the health effects of environmental tobacco smoke (ETS) on non-smokers, particularly the children of smoking parents[1,2]. Tobacco smoke contains many toxic compounds, which are distributed between the gas phase and the particulate phase[3–6]. ETS is an important source of total suspended particulate matter in homes and other indoor environments[7]. A strong correlation has been found between the mutagenicity associated with the particulate concentration in various homes and the number of cigarettes smoked[8].

Body fluids have been analyzed for nicotine and cotinine content as a means to assess personal exposure to ETS[9]. Personal air monitoring is an alternative approach to exposure assessment that is more direct and less intrusive than the analysis of body fluids. Moreover, it can be employed to assess exposures to ETS gases and particles separately.

To identify and quantitate the contribution of ETS to the total particulate mass in indoor environments, suitable marker (surrogate) compounds are required. Nico-

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tine has been used as a marker for ETS because it is unique to tobacco and is major component of tobacco smoke[10–12]. However, the suitability of nicotine as a marker for ETS particulate matter is uncertain because of unresolved questions regarding its reactivity and volatility and ambiguities regarding its phase distribution[13].

In the study reported here, another tobacco alkaloid, cotinine, was investigated as a potential marker compound for ETS particulate-phase exposure. Cotinine is less volatile than nicotine, occurs pimarily in the particulate phase, and can be measured with a nitrogen-specific detector for enhanced discrimination from other components of ETS. A literature search revealed no environmental applications of this compound. Raw extracts of particulate samples were analyzed by multidimensional gas chromatography (MDGC). By employing heart-cutting techniques and multiple columns of different polarities, MDGC provides enhanced GC separations and increased information content for the identification of cotinine and other analytes.

MATERIALS AND PROCEDURES

Equipment

Analyses were performed with a Siemens SiChromat-2 MDGC system containing two ovens operated with independent temperature programs and equipped with a flame ionization detection (FID) and a nitrogen-specific detection (NSD) system, also from Siemens. Sample injections were made with a Varian Model 1095 on-column capillary injector. Computing integrators (Perkin-Elmer, Model LC1-100) were employed to collect and process the data.

The sample pathway consisted of a retention gap/guard column, a pre-column, a short trapping column, and two parallel analytical columns connected to a single NSD system (Fig. 1). The pre-column effluents were switched automatically to either the FID or the trapping column by means of a Deans-type column-switching device. Effluents in the trapping column entered the two analytical columns through a glass-lined, stainless-steel splitter union (Alltech).

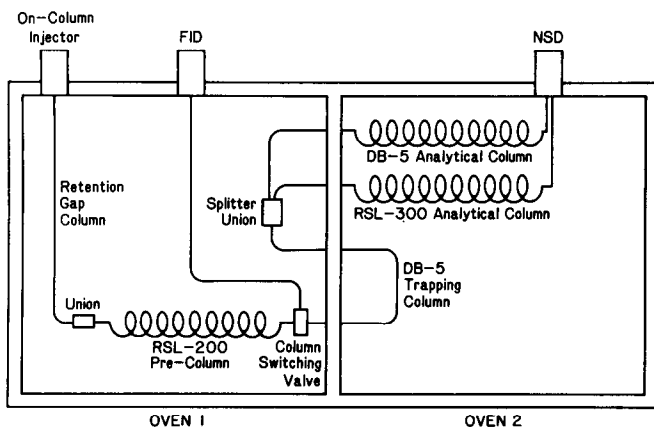The MDGC system was fitted with fused-silica, open tubular capillary columns.

Fig. 1. Schematic of the sample flow paths in the multidimensional gas chromatograph.

A length of uncoated, deactivated, fused-silica tubing (1 m × 0.32 mm I.D., J&W Scientific) served as a retention gap and protected the pre-column from any non-volatile compounds present in sample injections. The pre-column was an RSL-200 column, 15 m × 0.32 mm I.D. with 0.5 μm film thickness (Alltech). The trapping column was a short section of DB-5 column, 0.75 m × 0.32 mm I.D. with 1.0 μm film thickness. The analytical columns were (1) a DB-5 column, 12 m × 0.32 mm I.D. with 1.0 μm film thickness (J&W Scientific) and (2) an RSL-300 column, 15 m × 0.32 mm I.D. with 0.5 μm thickness (Alltech).

Materials

Nicotine calibration solutions were prepared from a reference standard solution obtained from Supelco and the cotinine solutions from 98% purity cotinine obtained from Aldrich. Calibration solutions were prepared in ACS-grade benzene (Fisher Scientific). Particulate extractions were carried out with chromatographic-grade methylene chloride (Burdick and Jackson).

Cigarette smoke particulate samples collected on 37-mm tetrafluoroethylene filters were obtained from the John B. Pierce Foundation. The samples had been collected from an environmental chamber containing several active smokers and were stored in a freezer until extracted. Blank samples were obtained from the chamber while it contained non-smokers.

Indoor air samples were obtained from Battelle-Columbus as concentrated extracts in ethyl acetate[14]. The samples were collected in private homes with a prototype indoor air sampler containing an XAD-4 sorbent bed and a105-mm quartz fiber filter in tandem.

Sample preparation

The cigarette smoke particulate sample filters were cut into strips and extracted in 30 ml methylene chloride with an ultrasonic probe (Heat Systems-Ultrasonics, Model W185). Extractions were carried out for 30 min at 30 watts. Extraction solutions were evaporated under a gentle stream of charcoal-filtered helium to approximate 1 ml, reconstituted with 1 ml benzene, and then evaporated to 0.5 ml. The concentrated solutions were mixed in an ultrasonic bath and centrifuged (*ca.* 400 *g*) prior to analysis.

The indoor air samples were received from Battelle-Columbus as concentrated extracts in ethyl acetate. The filter and XAD-4 samples had been combined and Soxhlet extracted with methylene chloride for 16 h and then further extracted with ethyl acetate for an additional 8 h. The two extracts were combined and concentrated by Kuderna–Danish evaporation. The concentrated solutions were analyzed as received.

Gas chromatography

High carrier gas flow-rates were used in the MDGC analyses to reduce retention times an minimize peak tailing. The linear flow-rate through the analytical columns was 29.6 cm/s at the initial programmed temperature settings. The NSD current was set at 800 mA to achieve maximum sensitivity. The nicotine and cotinine cuts from the pre-column were obtained over intervals of 0.35 min and 0.70 min, respectively. The two cuts were collected together on the trapping column with the oven set

at 50°C. The on-column injector was programmed from 60°C to 280°C at 100°C/min, the pre-column oven from 90°C to 280°C at 15°C/min, and the analytical oven from 50°C to 280°C at 15°C/min. The MDGC was programmed to reset automatically at the end of an analysis (30 min). It was ready for the next sample as soon as the injector had cooled (25 min). Sample injection volumes ranged from 1 μl to 3 μl. To reduce analyte losses to any active sites present in the injector or the retention gap column, 1 μl ammoniated benzene was co-injected with each sample.

Nicotine and cotinine were identified by their retention times on the two analytical columns. Confirmation was obtained by GC–matrix isolation–Fourier transform IR spectroscopic (FT-IR) analysis. Quantitation was based on peak height measurements of the chromatogram obtained with the DB-5 column.

RESULTS AND DISCUSSION

Validation of procedures

High cotinine recoveries were obtained in the extraction and concentration procedures. A 98% extraction efficiency was obtained for cotinine based on re-extractions of two ETS particulate samples. A 90% recovery (relative standard deviation, R.S.D. = 11%, n = 7) was obtained for cotinine from evaporated solutions of methylene chloride and benzene. The recovery of the more volatile nicotine was poorer (53%) and more variable (R.S.D. = 32%, n = 4).

Ammoniated benzene, produced by bubbling ammonia through the solvent, was co-injected with all samples to reduce losses of basic analytes in the MDGC system. NSD area responses for cotinine and nicotine obtained with co-injections of 1 μl benzene were compared to responses obtained with co-injections of 1 μl ammoniated benzene (Table I). Injections contained 0.9–2.0 ng of the analyte. Although co-injected ammoniated benzene had little effect on the cotinine response with new columns, it produced a substantial increase in response when employed with used columns (23% with both the DB-5 and the RSL-300 columns). A comparison of the results for cotinine and nicotine indicates that nicotine is more susceptible to adsorption losses in both new and used columns. Thus, some sample cleanup prior to analysis may be indicated to minimize buildup of active sites in the GC system.

Identification of analytes by chromatographic retention times requires precise and reproducible measurements. Replicate injections of solutions of cotinine at loadings between 0.5 and 4 ng yielded highly reproducible retention times on both the DB-5 and the RSL-300 columns. The standard deviations (S.D.) for six injections were 0.003 min and 0.004 min on the DB-5 and RSL-300 columns, respectively.

TABLE I

PERCENT INCREASE IN DETECTOR RESPONSES FOR COTININE AND NICOTINE RESULTING FROM CO-INJECTIONS OF 1 μl AMMONIATED BENZENE

Condition of guard column and pre-column	Cotinine		Nicotine	
	DB-5	RSL-300	DB-5	RSL-300
Used	23	23	722	679
New	2.4	1.7	6.6	9.2

In this study the retention times observed for unknown sample peaks were compared directly with the retention times obtained on the same day with standard samples yielding similar peak intensities. As a result, the identification of cotinine for all cigarette smoke and room air samples on both columns was based on retention times which agreed with a standard mixture to within 0.008 min. The agreements in retention times obtained on both columns, together with the quality of the NSD chromatogram (Fig. 2), permit unambiguous identification of the analyte peaks.

The NSD calibration data between 0.2 and 3.7 ng cotinine on the DB-5 column fit a linear regression line and showed a small non-zero intercept (Fig. 3). Further investigation revealed that the linearity did not extend below 0.2 ng. Calibrations conducted between 0 and 0.2 ng cotinine indicated a limit of detection (at three times noise) of 15 pg. The calibration curve with the more polar RSL-300 became non-linear below 1 ng, and the limit of detection was somewhat higher than that obtained with the DB-5 column. Consequently, the DB-5 chromatogram was selected for quantitation. Replicate sample injections yielded very reproducible peak heights for cotinine in the DB-5 chromatogram (R.S.D. = 1.3%, n = 8). As a result of the high reproducibility of the cotinine measurements, the high cotinine recoveries obtained in the sample extraction and concentration procedures, and the use of daily calibration standards, internal standards were not employed in this study.

Sample analysis

The NSD chromatograms of the combined cotinine and nicotine heart-cuts of the cigarette smoke particulate samples show that the analyte peak signals are well
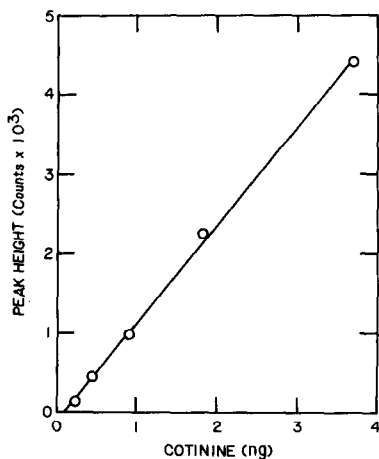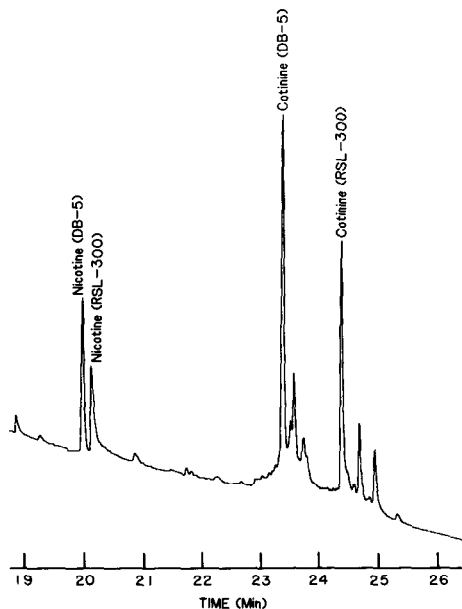

Fig. 2. NSD chromatogram of the combined nicotine and cotinine heart-cuts from the concentrated extract of an ETS particulate sample.

Fig. 3. Cotinine calibration for the DB-5 column with the nitrogen-specific detector.

TABLE II
ANALYSIS OF FILTER AND XAD-4 COMBINED EXTRACTS FROM THE LIVING ROOMS OF
PRIVATE RESIDENCES

Sample	Smoking rate (cigarettes/h)	Concentration in room air (ng/m³)	
		Cotinine	Nicotine*
4-L	1.9	316	29 000
3-L	1.3	21	1 700
8-L	0	5	60

* Nicotine analyses performed by the Battelle-Columbus laboratory[14].

separated and easily distinguished on both the DB-5 and RSL-300 columns (Fig. 2). Moreover, the analytical procedure requires only one detector for both analytical columns. The particulate-phase concentration of cotinine was determined in samples from two chamber studies conducted two years apart, in which five popular brands of cigarettes were smoked. The average concentration of cotinine in the six ETS particulate samples was 732 ng/μg. The consistency of this value (S.D. = 83) suggests that cotinine may be a suitable candidate marker compound for cigarette smoke particulate mass. No cotinine was detected in a filter blank obtained with the environmental
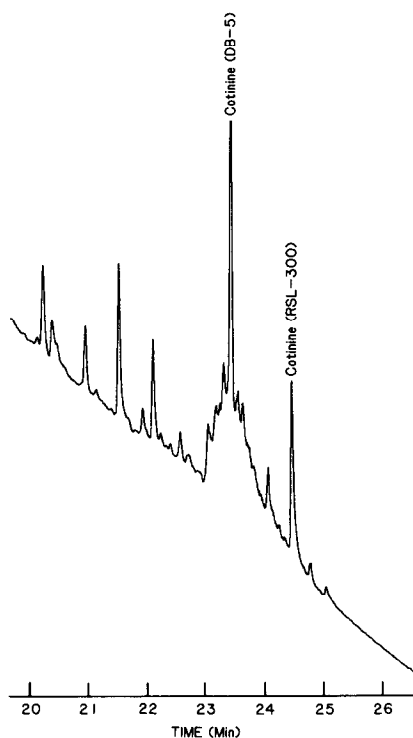

Fig. 4. NSD chromatogram of the cotinine heart-cut from the concentrated extract of living room air sample 3-L. The baseline drift is apparently the result of the rising temperature of oven 2. The unmarked peaks have not been identified.

chamber occupied by several non-smokers. Although nicotine was found in all of the samples, volatility losses precluded meaningful quantitative measurements. However, previous nicotine results were consistent for cigarette smoke samples collected on sodium bisulfate-treated filters[11].

Data for air samples from the living rooms of three homes that were analyzed for cotinine are shown in Table II and Fig. 4. Although these samples are composed of both the vapor-phase and particulate-phase cotinine, studies have shown that cotinine occurs mainly in the particulate phase[15,16]. The cotinine peaks illustrated in Fig. 4 were obtained from a sample collected in the home reporting a smoking rate of only 1.3 cigarettes/h. The injected sample contained 371 pg cotinine. Since the limit of detection of cotinine with the MDGC is 15 pg, it appears that the detection and measurement of cotinine in indoor particulate matter can be accomplished for smoking rates about one order of magnitude less than that represented in sample 3-L.

Although the cotinine concentration was found to increase with smoking rate, the increase is not proportional. Since the nicotine and cotinine concentrations are fairly proportional to one another, the cotinine results do not appear to be artifacts of the MDGC analysis. The large difference in concentration between samples 4-L and 3-L probably reflects differences in the ventilation rates of the two homes during sampling. The presence of cotinine and nicotine in homes reporting no smoking (8-L) suggests the possible presence of residue from smoking sometime previous to the sampling.

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