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Isotope dilution gas chromatography–mass spectrometry in the determination of benzene, toluene, styrene and acrylonitrile in mainstream cigarette smoke

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SUMMARY

A cryogenic trapping method with isotope dilution gas chromatography–mass spectrometry analysis has been developed for the determination of benzene, toluene, styrene and acrylonitrile in mainstream vapor phase cigarette smoke. The method is simple, direct, and quantitative. Vapor phase samples are collected cryogenically in a series of four traps following removal of the particulate phase with a Cambridge filter pad. For all four analytes, 75–85% of the total amounts recovered were found in the initial trap and less than 1% in the final trap. Assessment of instrumental precision by multiple injections of a sample gave relative standard deviations of less than 2%. Linear calibration for all analytes over the analysis range gave an r^2 value greater than 0.99 with average relative standard deviations at the mean ranging from 1.4 to 8.2%. The cigarettes analyzed include a reference cigarette (Kentucky 1R4F), a commercial ultra-low "tar" mentholated cigarette, and two cigarettes that heat but do not burn tobacco. The values determined for the four analytes in the 1R4F samples are comparable to reported values of similar cigarettes. The cigarettes which heat rather than burn tobacco yield less of all four analytes compared to the other cigarettes in the study.

INTRODUCTION

The determination of volatile organic compounds in cigarette smoke is challenging due to the complexity of the smoke matrix[1]. With the advent of low and ultra-low tar brands, yields of volatile organics are generally on the order of micrograms per cigarette. The recent development of a cigarette brand that heats but does not burn tobacco[2] presents a different type of smoke matrix for which precise and accurate smoke composition data are also useful.

The determination of minor smoke components often requires a concentration step prior to instrumental analysis because cigarette smoke is a dilute, complex mixture consisting mainly of air[1]. Two approaches widely used for this purpose are

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cryogenic trapping[3–7] and sorption–desorption methods with a solid substrate such as Tenax[8–10].

Cryogenic trapping has been used historically in the analysis of vapor phase cigarette smoke to collect samples under conditions of low reactivity[3–5]. This minimizes sample degradation which is an important concern for quantitative measurements of analytes at low concentrations. Cryogenic methods using two different types of cold traps have been reported[6,7]. A glass trap submerged in liquid nitrogen has been used to collect whole smoke from a domestic filter blend cigarette with quantitative analysis by gas chromatography (GC)[6]. A recent report has demontrated that vapor phase smoke can also be sampled directly from individual puffs and trapped on a cold capillary column for subsequent GC–mass spectrometric (MS) analysis[7].

Vapor phase components from smoke can be trapped on Tenax and thermally desorbed onto a gas chromatograph for analysis[8]; however, Tenax has a low loading capacity for highly volatile organics[9] and some breakthrough has been noted of highly volatile smoke components from the Tenax trap from cigarettes that have higher tar yields[8]. In addition, an undesirable high-temperature desorption step is required to release the analytes from Tenax. Tenax can contribute background response to some analytes of interest[10] which ultimately affects both precision and accuracy.

Our objectives in this work were to develop a method that could determine selected smoke components from different types of cigarettes and which could accommodate a wide range of analyte concentrations. To meet these objectives we combined cryogenic trapping of cigarette smoke with isotope dilution GC–MS. Cigarette smoke is trapped in methanol at $-70°C$ and samples are analyzed without additonal concentration or purification steps. The simplicity of the procedure favors quantitative analysis because potential losses from chemical reaction, analyte decomposition, and non-quantitative transfer during extensive chemical fractionation are minimized. Operating the mass spectrometer in the selected-ion monitoring mode virtually eleminates background contributions and the use of isotopically labelled analogs as internal standards (isotope dilution) provides a more precise and accurate method for quantifying the trapped analytes than external standard or conventional internal standard methods[11]. Isotopically labelled analogs compensate for potential losses during sample transfer and for instrumental variability because they have physical, chromatographic, and mass spectral properties that are nearly identical to those of the analytes.

In this study the method is applied to low-tar and ultra-low-tar cigarettes, and to cigarettes that heat but do not burn tobacco. The analytes determined are acrylonitrile, benzene, toluene and styrene. These compounds are all associated with the vapor phase of cigarette smoke and have been previously reported in cigarette smoke[11–13].

EXPERIMENTAL

Cigarettes

Four different cigarettes were analyzed in this study. The cigarettes included the 1R4F reference cigarette produced by the Tobacco and Health Research Institute (Lexington, KY, U.S.A.) and a commercial ultra-low-tar mentholated brand (ciga-

rette A). Two cigarettes that heat rather than burn tobacco were analyzed, one regular (cigarette B) and one mentholated (cigarette C).

Chemicals

Acrylonitrile, benzene, toluene and styrene were obtained from Aldrich (Milwaukee, WI, U.S.A.). [$^2H_8$]Styrene and [$^2H_3$]acrylonitrile were obtained from Cambridge Isotope Labs. (Woburn, MA, U.S.A.). [$^2H_6$]Benzene and [$^2H_8$]toluene were obtained from MSD Isotopes (Montreal, Canada). The purities of all isotopically labelled materials were 98 atom% ^2H or greater. Methanol was high-purity solvent grade obtained from American Burdick and Jackson (Muskegon, MI, U.S.A.).

Solutions

A primary stock solution of each analyte was prepared by accurately weighing into a 10-ml volumetric flask 100 μl of the neat analyte. Each solution was diluted to the mark with methanol and mixed well. A secondary stock solution was prepared by adding the following volumes from the primary stock solutions into one 10-ml volumetric flask: 0.400 ml acrylonitrile, 1.00 ml benzene, 1.00 ml toluene and 0.100 ml styrene. The solution was diluted to the mark with methanol and mixed well.

Stock solutions of [$^2H_3$]acrylonitrile and [$^2H_8$]styrene were prepared by weighing accurately into two 10-ml volumetric flasks 100 μl of each neat material, respectively. Each solution was diluted to the mark with methanol and mixed well. An internal standard spiking solution was prepared by adding 1.00 ml of the [$^2H_3$]-acrylonitrile stock solution and 0.400 ml of the [$^2H_8$]styrene stock solution to a 10-ml volumetric flask and by accurately weighing 50 μl of [$^2H_6$]benzene and 50 μl of [$^2H_8$]toluene to the flask. The solution was diluted to the mark with methanol and mixed well.

Four standard solutions were prepared by adding 100, 500, 1500 and 3000 μl of the secondary stock solution to four respective 10-ml volumetric flasks. A volume of 100 μl of the internal standard spiking solution was added to each flask. The solutions were diluted to the mark with methanol and mixed well. All solutions were stored at 4°C and allowed to warm to room temperature before use.

Smoke generation and collection apparatus

Mainstream vapor phase smoke was isolated by using the apparatus shown in Fig. 1. Cigarettes were smoked on a Model RM20/CS 20-port Heinrich Borgwaldt

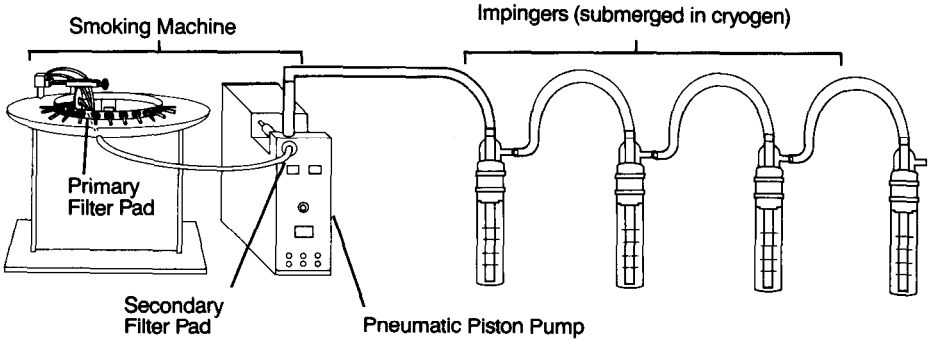

Fig. 1. Apparatus used for the collection of mainstream vapor phase cigarette smoke.

rotary smoking machine (Heinrich Borgwaldt, Hamburg, F.R.G.). The mainstream smoke was passed through a central Cambridge filter pad to remove particulate phase matter and through a secondary filter pad to ensure that no breakthrough of the particulate phase occured. All connections between the filter pads, the pneumatic piston pump, and the impingers were made with 0.25-inch Tygon tubings which had been previously rinsed with methanol. The effluent from the pneumatic piston pump was passed through four Midget Impingers (Ace Glass, Vineland, NJ, U.S.A.) connected in series. The impingers were modified to eliminate the constricted opening of the inlet tube and to extend the inlet tube length to within 1 mm of the bottom of the container. A 5-ml volume of methanol was placed in each impinger along with approximately 5 g of 3-mm glass beads in order to raise the level of methanol and to increase the cold surface area. The impinger joints were wrapped with Parafilm™ to effect an airtight seal. Each impinger was submerged in an isopropanol–dry ice cryogenic bath (−70°C).

Smoking procedure and sample collection

All cigarettes were smoked on the apparatus described above according to the Federal Trade Commission (FTC) puffing regimen (one 35 ml puff of 2 s duration every 60 s). The cigarettes were lit with a hydrogen flame. The 1R4F and the ultra-low-tar cigarettes were smoked to a butt length of 3 mm from the filter overwrap. Cigarettes B and C were smoked until the heat source was completely consumed. The butt length does not change during smoking of these cigarettes, and 9–10 puffs is their standard FTC smoking activity. Smoke was collected from 80 cigarettes for each sample except for the 1R4F cigarettes. Twenty 1R4F cigarettes were smoked per sample because of the relatively high analyte concentrations in the 1R4F mainstream vapor phase. For 1R4F cigarettes six samples were collected and analyzed. Three samples were collected and analyzed for cigarettes A, B and C.

When the smoking process described above was completed, 50 μl of the internal standard spiking solution were immediately added to each impinger. The impinger was capped with a solid stopper and vigorously shaken for 1 min with occasional venting. Samples of the impinger contents were transferred to GC vials and sealed with crimp caps.

Sample blanks were collected by inserting a cigarette filter into the smoking machine and dry puffing for an equivalent of 80 cigarettes.

GC–MS analysis

The GC–MS system used was a Hewlett-Packard HP 5970B MSD (Hewlett-Packard, Palo Alto, CA, U.S.A.) coupled to an HP 5890 GC via an open-split interface. The mass spectrometer was tuned by using perfluorotributylamine prior to analyzing a series of samples (every 2–3 days). An HP 7673 automatic liquid sampler was used to inject 1 μl of sample in the splitless mode (splitless time = 0.5 min). Analytes were separated on a J&W DB1-60W, 5.0-μm film, fused-silica capillary column (J&W Scientific, Folsom, CA, U.S.A.) by using helium as carrier gas at a head pressure of 22.5 p.s.i.g. The temperatures for the injection port and transfer line were 220°C and 250°C, respectively. For each analysis the GC oven was held at 35°C for 10 min. and then heated at 3°C/min to a temperature of 166°C. After elution of the analytes of interest, the column was heated at a rate of 50°C/min to 230°C and held for 5 min to clear the column of late eluting material.

The mass spectrometer was operated in the selected-ion monitoring mode. A separate chromatographic time window was used to monitor each analyte and its internal standard. Three ions each were monitored for the analyte and the internal standard (Table I). The scan frequency was 2.0 Hz. Each analyte and labeled internal standard was identified by retention time and the relative concordant responses of the multiple ions monitored. Only the molecular ions of each analyte and its internal standard were used for quantitation.

TABLE I

IONS MONITORED FOR EACH ANALYTE AND INTERNAL STANDARD

Ions shown in bold were used for quantitation.

Compound	*Internal standard*	*Ions monitored*
Acrylonitrile		26, 52, **53**
	[$^2H_3$]Acrylonitrile	26, 54, **56**
Benzene		39, 52, **78**
	[$^2H_6$]Benzene	42, 56, **84**
Toluene		39, 78, **92**
	[$^2H_8$]Toluene	42, 84, **100**
Styrene		39, 78, **104**
	[$^2H_8$]Styrene	42, 84, **112**

Response factors were determined daily by analyzing the series of four standard solutions. Quantitation was performed for each trap by using the method of internal standards. The total amount of the analyte per sample collection was obtained by summing the averages of duplicate injections across all four traps.

RESULTS AND DISCUSSION

The method was assessed with regard to trapping efficiency, instrumental precision, and chromatography.

Trapping efficiency

Fig. 2 is a plot of the amount of analyte per trap relative to the total amount in all the traps for the 1R4F and cigarette B. More than 75% of each compound was found in the first 1R4F trap and less than 1% was found in the final trap. With cigarette B, a greater percentage (more than 85%) was observed in the first trap presumably because less material was produced from this cigarette even though four times as many cigarettes were smoked as for the 1R4F. The last two traps show no analytes present for cigarette B. The diminishing amount in sequential traps for both cigarettes demonstrates excellent collection of the analytes in this study.

Instrument precision and linearity

Instrumental precision was assessed by replicate injections of both a low-con-

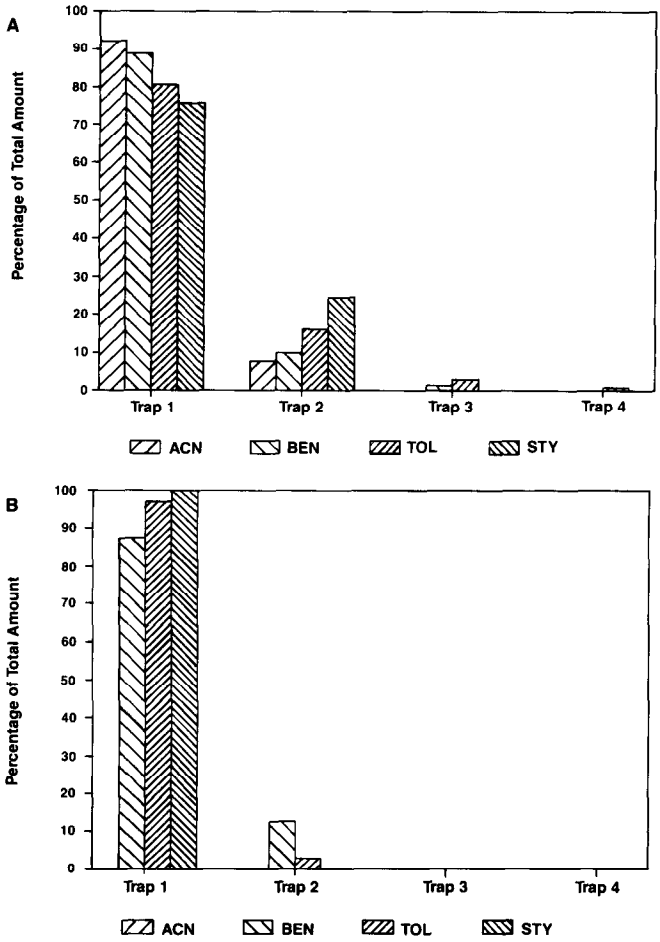

Fig. 2. Trapping efficiency plots for (A) 1R4F and (B) cigarette B samples. ACN = Acetonitrile; BEN = benzene; TOL = toluene; STY = styrene.

centration standard solution and a trap 1 sample from a ultra-low-tar cigarette. Average area response ratios and relative standard deviations (R.S.D. values) observed for each compound are summarized in Table II. Instrumental precision is comparable when estimated with either the standard solution or the smoke sample. R.S.D. values of 1.2% or less are observed for all compounds in the former case and R.S.D. values of 2% or less are observed for the latter. Instrument response linearity was determined from calibration plots of the standard solutions. In all cases, the r^2 value was greater than 0.99 for each analyte. The average R.S.D. values of the predicted values at their means were 2.4, 6.2, 8.2 and 1.4% for acrylonitrile, benzene, toluene and styrene, respectively[14].

TABLE II

AVERAGE AREA RESPONSE RATIOS OF UNLABELLED TO LABELLED $M^{+\cdot}$ FOR FIVE REPEATED INJECTIONS OF A LOW-CONCENTRATION STANDARD SOLUTION AND TRAP 1 OF AN ULTRA-LOW-TAR CIGARETTE

S.D. is the standard deviation and R.S.D. is the relative standard deviation.

Compound	*Standard solution*		*Ultra-low-tar cigarette*	
	Average ± S.D.	*R.S.D. (%)*	*Average ± S.D.*	*R.S.D. (%)*
Acrylonitrile/ [$^2H_3$]acrylonitrile	0.411 ± 0.004	0.9	1.79 ± 0.04	2.1
Benzene/ [$^2H_6$]benzene	0.335 ± 0.001	0.2	4.38 ± 0.01	0.3
Toluene/ [$^2H_8$]toluene	0.230 ± 0.001	0.2	3.50 ± 0.01	0.3
Styrene [$^2H_8$]styrene	0.298 ± 0.004	1.2	1.61 ± 0.01	0.7

Chromatography

The non-polar column used in this work provided good resolution of all analytes. Typical chromatograms for acrylonitrile and a representative aromatic compound (styrene) are shown in Fig. 3 and 4. The acrylonitrile peak shape was broader and exhibited increased tailing compared to those of the aromatic compounds. As such, the method sensitivity and presicion for acrylonitrile were reduced relative to the other compounds studied. The limit of quantitation for acrylonitrile, defined as the concentration of acrylonitrile in the lowest response factor standard analyzed, was 0.2 μg/cigarette. As can be seen in Fig. 3B, the trace for m/z 53 shows a slight positive response for cigarette B. However, this response was below the limit of quantitation and too weak to determine if this response was due to acrylonitrile or an interference. All other compounds exhibited sharp, well-defined peaks with no observable background interference. The limits of quantitation for the aromatic compounds were 0.05 μg/cigarette.

Quantitative data

The measured amounts of each analyte and wet total particulate material (amount of material retained on the Cambridge filter pad) for each cigarette are summarized in Table III. R.S.D. values ranged from 3 to 27% with most being less than 15%. These results indicate that the sampling variability, which includes inherent cigarette variability and smoke collection variability, is greater than the instrumental variability described above. The amount of acrylonitrile determined in 1R4F smoke (7.6 μg/cigarette) falls in the range of reported values (3.2–15 μg/cigarette)[13]. The aromatic compound concentrations found in the 1R4F smoke also agree well with reported values (Table IV). Determination of benzene and toluene in mainstream vapor phase smoke by the procedure of Brunnemann *et al.*[17] for cigarettes that heat but do not burn tobacco shows fair agreement with our results for toluene

Fig. 3. Representative selected ion chromatograms for acrylonitrile and [$^2H_3$]acrylonitrile from trap 1 of samples (A) 1R4F and (B) cigarette B. D_3-Acrylonitrile = [$^2H_3$]Acrylonitrile.

Fig. 4. Representative selected-ion chromatograms for styrene and [$^2H_8$]-styrene from trap 1 of samples (A) 1R4F (B) cigarette B. D_8-Styrene = [$^2H_8$]Styrene.

TABLE III

AMOUNTS OF ANALYTES DETERMINED IN MAINSTREAM VAPOR PHASE SMOKE

Cigarette[a]	*WTPM*[b] *(mg/cigarette)*	*Analyte (μg/cigarette)*[c,d]			
		Acrylonitrile	*Benzene*	*Toluene*	*Styrene*
1R4F	11.4	7.6 ± 0.8	45 ± 3	68 ± 4	2.1 ± 0.3
A	1.5	0.6 ± 0.1	6.7 ± 0.2	5.9 ± 0.6	0.11 ± 0.03
B	9.0	<0.2	1.9 ± 0.1	0.46 ± 0.04	0.077 ± 0.007
C	9.4	<0.2	2.1 ± 0.1	0.7 ± 0.1	0.051 ± 0.008

[a]Cigarette A is a commercial ultra-low-tar mentholated brand; B is a cigarette that heats rather than burns tobacco; C is the mentholated version of B.

[b]Wet total particulate matter.

[c]The '±' number represents one standard deviation.

[d]Blank determinations yielded no measurable amount of any analyte.

(0.4 μg/cigarette) but a much lower value for benzene (0.3 μg/cigarette). The cause of this discrepancy may be variations in the lighting technique for these types of cigarettes.

Of the cigarettes studied, the 1R4F smoke contained the greatest concentrations of each analyte. Cigarette A has a wet total particulate matter (WTPM) that is 87% less than that of the 1R4F and, correspondingly, all of the analytes in A are reduced relative to the 1R4F by 85–95%. However, the WTPM values for cigarettes B and C (those that heat rather than burn tobacco) are similar to that of the 1R4F but still show more than 90% reduction of the analytes relative to 1R4F. Even if acrylonitrile had been detected in cigarettes B and C at its quantitation limit of 0.2 μg/cigarette, a reduction of 97% relative to 1R4F would have been observed. The analyte reductions for cigarettes B and C relative to both the 1R4F and the ultra-low-tar

TABLE IV

COMPARISON OF RESULTS FOR AROMATIC COMPOUNDS IN THE 1R4F WITH OTHER REPORTED VALUES

Compound	*μg/cigarette*		
	This work	*Brunnemann et al.*[a]	*Higgins et al.*[b]
Benzene	45	51	42
Toluene	68	73	55
Styrene	2.1	–	3

[a]Values for 1R4F[7]. The 1R4F yields 9.2 mg tar under FTC conditions.

[b]Values for a filtered American commercial brand with a 7 mg tar yield[8].

cigarette A suggest a simpler smoke chemistry for these cigarettes. This is not surprising since heating instead of burning tobacco would be expected to yield a less complex smoke. The visual appearance of the samples also supports this premise. Trap 1 samples from the Kentucky reference 1R4F and the ultra-low-tar cigarette A were slightly discolored while comparable samples from cigarettes B and C were virtually colorless.

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