Journal of Chromatographic Science 2012;50:387–392
doi:10.1093/chromsci/bms016 Advance Access publication March 8, 2012

Article

Determination of Seven Nitrobenzene Compounds in Mainstream Cigarette Smoke with Heart-Cutting Two-Dimensional Gas Chromatography

Fuwei Xie[1,2], Jingjing Shang[2], Jizhao Guo[2], Zhao Ge[2] and Shusheng Zhang[1*]

[1]Chemistry Department of Zhengzhou University, Zhengzhou 450052, P.R. China, and [2]Zhengzhou Tobacco Research Institute of CNTC, Zhengzhou 450001, P.R. China

*Author to whom correspondence should be addressed. Email: zhangshusheng@371.net

Received 31 January 2011; revised 12 May 2011

A heart-cutting two-dimensional gas chromatography (GC) method was developed for the determination of nitrobenzene compounds (NBCs) in mainstream cigarette smoke. For the method, the particulate matter of cigarette smoke was extracted with cyclohexane, purified with a silica solid-phase extraction (SPE) cartridge and analyzed by heart-cutting two-dimensional GC equipped with two electron capture detectors. The heart-cutting two-dimensional GC was achieved by a single-column GC oven equipped with a microfluidic pressure balanced device (Deans switch). Two-dimensional GC was compared to single-dimensional GC and found to be clearly better for the separation of seven NBCs from a complex smoke matrix. The limits of detection ranged from 1.28 to 9.83 ng/mL, spiked recoveries were between 88.3 and 106.8% and relative standard deviation ranged from 2.79 to 12.78%. The NBCs yields of six kinds of Chinese and international cigarettes brands, which were all smoked according to two smoking protocols (International Organization for Standardization and Health Canada Intense smoking regimens), were determined and compared.

Introduction

Nitrobenzene compounds (NBCs), which are readily absorbed through the skin and inhaled by respiration, may cause serious damage to the central nervous system and cause liver damage, anemia and lung irritation (1). The International Agency for Research on Cancer (IARC) has classified nitrobenzene as a Group 2B carcinogen, meaning that it is possibly carcinogenic to human beings (2–4). Smoking cigarettes will produce NBCs that can be inhaled by smokers. In 1970, Hoffmann developed a gas chromatography (GC) method for the determination of the eight NBCs in nonfiltered cigarette smoke (5). Since then, no other works about NBCs in cigarette smoke have been reported, and all literature related to NBCs in cigarette smoke have cited the data from Hoffmann's works (6–9). In Hoffmann's experiment, a packed column was used for GC separation and sample preparation was very complex [for example, multiple steps were involved such as water-stream distillation, liquid-liquid extraction (LLE), cleanup with alumina column], which might decrease the accuracy of the analysis results. On the other hand, during recent decades, scientific technologies have developed and the cigarette has changed greatly. Therefore, a simple, sensitive and accurate method is required for the reanalysis of NBCs in cigarette smoke.

Because the contents of NBCs in cigarette smoke are as low as several nanograms per cigarette, determination of NBCs in cigarette smoke is a challenging task. Currently, two-dimensional GC system has been rapidly developed to solve the dilemma encountered by single-column GC for analysis trace targets in very complex matrixes (10–18). Heart-cutting two-dimensional GC transfers selected cuts from one column to another with different properties so that the selectivity of the separation and the resolution of the peak groups that are contained in such cuts is highly improved. Consequently, owing to the complex composition of cigarette smoke and the low yields of NBCs, a combination of SPE and heart-cutting two-dimensional (2D) GC was chosen to analyze the NBCs in mainstream cigarette smoke. Therefore, the aims of the present work are to: (1) develop a practical collection, extraction and cleanup procedure prior to the analysis of seven NBCs (Figure 1) in cigarette smoke [because the commercial reference compound was not available, 2-nitro-1, 4-dimethylbenzene, reported by Hoffmann (5), was not included in this study]; (2) develop a rapid and sensitive method to the analysis of seven NBCs; (3) determine the seven NBCs in actual cigarette samples by the developed 2D-GC.

Experimental

Reagents and chemicals

The following seven NBCs were used in this study: nitrobenzene (99%), 2-nitrotoluene (99%), 3-nitrotoluene (99%), 4-nitrotoluene (99%) (used as internal standard), 4-nitro-*o*-xylene (99%); all supplied by Dr. Ehrenstorfer (Schlosser, Germany); 4-nitro-*m*-xylene (99%) was purchased from Sigma-Aldrich (Milwaukee, WA), 4-ntrocumene (99%) was made available by TCI (Tokyo, Japan). All organic solvents (methanol, cyclohexane and dichloromethane) were of high-performance liquid chromatography grade and obtained from J.T. Barker (Phillipsburg, NJ). Stock standard solutions (1 mg/mL) of each compound were prepared in cyclohexane, stored and refrigerated at 4°C. These stock standard solutions were diluted with cyclohexane to prepare a mixed stock of NBCs so that the concentration of mixture was 10 mg/mL nitrobenzene, 9.8 mg/mL 2-nitrotoluene, 10 mg/mL 3-nitrotoluene, 10 mg/mL 4-nitro-*o*-xylene, 10 mg/mL 4-nitro-*m*-xylene and 10 mg/mL 4-ntrocumene, respectively. Working standard solutions were then freshly prepared by diluting the mixed standard solution with cyclohexane to the required concentrations. Stock and working standards were stored at 4°C.

Apparatus

Separations were performed on a 6890N GC (Agilent Technologies, Wilmington, DE) equipped with a split/splitless

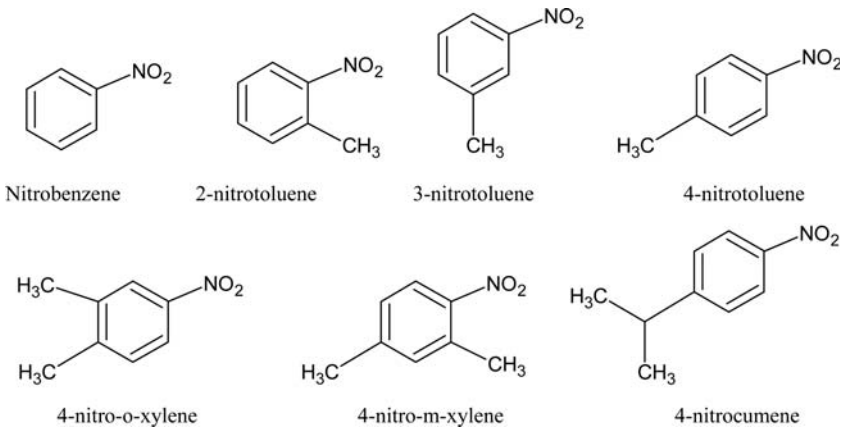

Figure 1. Structure of seven NBCs.

inlet, a pneumatics control module (PCM), two ECDs, an Agilent 7683B automated liquid sampler and a Deans switch kit. This kit consists of microvolume Hastelloy C tees with special fused-silica column adapters (VICI, Houston, TX), inert Silcosteel tubing (Restek, Bellfonte, PA) and other hardware and software (Agilent Technologies).

A Borgwaldt-KC RM 20H rotary smoking machine (HB, Germany) was used as the smoking engine for puffing the cigarette. A TurboVap II Concentration Evaporator Workstation (Zymark, Hopkinton, MA) was used for concentration. A KQ-700DB ultrasonic bath (Kunshan Ultrasonic, China) was used for extraction.

Smoke collection

The cigarettes used for this study were separately conditioned and smoked following the International Organization for Standardization (ISO) and Health Canada Intense (HCI) smoking protocols. The ISO3308 smoking protocol specifies that cigarettes whose ventilation holes are not blocked must be puffed with a 35-mL puff volume with one puff every 60 s and a 2-s puff duration, and 20 cigarettes must be smoked per cycle. The HCI smoking condition is as follows: 55 mL puff volume, 60 s puff interval, 2 s puff duration and 100% blocking of ventilation holes, and 10 cigarettes were smoked on 10 alternate ports of the RM 20H rotary smoking machine per cycle. As specified by ISO and HCI regimens, the Cambridge filter pad (CFP) and test cigarettes were conditioned for a minimum of 48 h at 22 $\pm$ 1°C and a humidity of 60 $\pm$ 2% before use.

The total particulate matter of smoke was collected on a 92-mm diameter CFP. Eighty cigarettes were smoked to produce enough NBCs when smoking according to the ISO3308 smoking protocol. After smoking, all CFPs were collected and extracted with the method described in the following section. Because of the higher yields of NBCs in cigarette smoke, only 20 cigarettes were needed when using the HCI regimen.

Sample extraction and clean-up

CFPs that collected cigarette smoke were extracted by ultrasonication for 30 min at room temperature in an Erlenmeyer flask with 160 mL of cyclohexane and 80 μL internal standard (4-nitrotoluene, 10 μg/mL) added. After extraction, five fractions of extract (20 mL each) were loaded onto five separate SPE silica cartridges of 500 mg/3 mL capacity (each cartridge was preconditioned with 10 mL of methanol followed by 10 mL of cyclohexane). The loaded samples were eluted with 10 mL of cyclohexane after all the samples had run through the cartridges at a 1 mL/min flow-rate. Five effluents of sample and corresponding eluent were combined and the total effluent volume of the five SPEs was reduced to 1 mL using a stream of nitrogen at 40°C. The resulting concentrate was analyzed by the heart-cutting 2D-GC.

2D-GC analysis

Samples were analyzed on an Agilent 6890N GC equipped with a Deans switch and two m-ECD detectors. The GC conditions were as follows: the Deans switch method was run in constant pressure mode. The inlet pressure was 40.10 psig, giving a flow of 2 mL/min for Column 1. The PCM pressure was set to 29.4 psig to give a flow of 3 mL/min to Column 2. The injection was set to splitless mode at 230°C and the injection volume was 1 mL. Both m-ECD temperatures were 300°C. For different NBCs, different GC oven temperature programs were used and only one cut was done per run. For nitrobenzene, 4-nitro-*o*-xylene, 4-nitrotoluene and 4-ntrocumene, the GC oven was initially held at 60°C for 2 min, raised to 100°C at a rate of 5°C/min, held for 10 min, increased to 170°C at a rate of 2°C/min, raised to 240°C at a rate of 20°C/min and held at 240°C for 10 min. For 3-nitrotoluene and 4-nitro-*m*-xylene, the GC oven was temperature-programmed as follows: 60°C for 2 min, 2°C/min ramp to 120°C (held for 10 min), 2°C/min ramp to 150°C and 20°C/min ramp to 240°C (held for 10 min). For 2-nitrotoluene, initial temperature was 60°C (held for 2 min), followed by increase at 2°C/min to 104°C (held for 10 min), then at 2°C/min to 140°C, and finally at 20°C /min to 240°C (held for 10 min). Concerning the column system, a 30 m $\times$ 0.25 mm i.d., 0.25-mm film DB-5MS (Agilent Technologies) was used for Column 1, and a 30 m $\times$ 0.25 mm i.d., 0.25 mm film HP-INNOWAX (Agilent Technologies) was used for Column 2. The restrictor tubing was 0.786 m with internal diameter of 0.1 mm. Helium was used as the carrier gas. The makeup gas for m-ECD detectors was nitrogen with a flow-rate of 60 mL/min.

Qualitative analysis was carried out by comparing the retention time with standards and highlighting the peak of target

substances by adding standards. Quantitative data of NBCs were obtained by the internal method using the calibration curves.

Results and Discussion

Optimization of extraction and SPE clean-up procedure

Cyclohexane and dichloromethane were tested to select the appropriate extraction and elution solvent. Suitable amounts of standards were added to blank CFDs and applied directly to the SPE silica cartridges after extraction with cyclohexane. SPE cartridges with added standards were eluted with 0, 5, 10 and 15 mL cyclohexane, respectively, and the result showed that all the NBCs were eluted out with 10 mL cyclohexane. In a similar manner, the extraction and elution patterns with dichloromethane were also examined. The recoveries for the seven NBCs, cyclohexane and dichloromethane were identical. However, when dichloromethane was used as extraction and elution solvent, there were many more interferents in the cigarette smoke extraction. Therefore, cyclohexane was selected as extraction and elution solvent in this investigation.

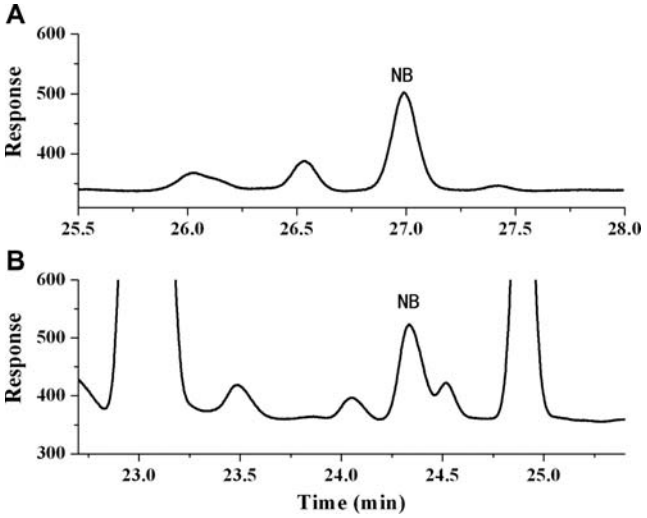

Figure 2. 2D chromatogram of cigarette smoke spiked with nitrobenzene (NB) standard using (A) DB-5MS and (B) DB-17 as the first column.

Optimization of chromatographic conditions

Column selection

The primary and secondary column phases were chosen to have different selectivity. The analytes of interest were separated on the first column from at least the bulk of the sample matrix. The analytes and any co-eluting matrix interference were then cut onto the second column. Because the phase of the second column had a different selectivity, the analytes were (hopefully) separated from the matrix interference. For general analytical separations, components were separated in boiling point order on the first column, and then a second separation was made, primarily based on the polarity of the analytes. An HP-INNOWAX column was selected for the 2D separation. Lower polar columns, a DB-5MS and a DB-17, were tested on the one-dimensional (1D) chromatograph.

It was found that, for the separations of seven possible NBCs in the mainstream cigarette sample, the combination of the DB-5MS and HP-INNOWAX columns was basically identical to or better than the combination of the DB-17 and HP-INNOWAX columns (Figure 2 shows 2D separation of nitrobenzene as an example). Comparison of the two chromatograms in Figure 2 shows that when the DB-17 column was used as the first column, nitrobenzene in the cigarette smoke interfered with the other compounds after being separated on the second column. However, when the DB-5MS column was used as the first column, a better separation of nitrobenzene was obtained. Compared to the DB-17 column, the DB-5MS and HP-INNOWAX columns had larger differences in polarity, which may increase the peak capacity of the 2D chromatogram. Therefore, the combination of the DB-5MS and HP-INNOWAX columns was preferable for sample separation.

Column flow rates

In a Deans switch method, the flow from the primary column plus a switching flow goes through the secondary column. The primary column flow is usually chosen to be at or near the optimum for that column. This prevents the flow through the secondary column from exceeding the optimum to such an extent that there is a significant loss in resolution. The flow for the secondary column is determined by taking the flow from the primary column and increasing it by approximately 50%, with a minimum increase of 1.0 mL/min. The difference between the two flows reflects the switching flow, which must be large enough to prevent tailing in the switch. According to Agilent manual G2855–90120 (19), two groups of flow rates were investigated at the initial oven temperature: (1) 1 mL/min for the first column and 2 mL/min for the second column; (2) 2 mL/min for the first column and 3 mL/min for the second column. The results showed that the second group of flow rates could supply a larger signal-to-noise ratio (because ECD is a concentration-type detector whose response is proportional to concentration) and faster chromatograms with only a small reduction in chromatographic resolution.

Oven temperature program

Experiments proved that if each two NBCs were cut and determined using the same oven temperature program, components from one cut interfered with those NBCs from another due to the complex matrix of the mainstream cigarette smoke. To avoid interference between two cuts, only one target compound had to be cut per run, and it was desirable to adopt different oven temperature programs for different target compounds. Thus, the different oven temperature programs were optimized for each target compound, as shown in the "Experimental" section.

Optimization for the switching times

The selection of suitable cut times is very important to heart-cutting 2D-GC. Cutting the time short may result in loss of the target, while a lengthy switching time may transfer relatively more interferents to the second column, which will affect the separating result of the target.

To obtain appropriate cut times, a standard was prepared that contained the highest concentration of seven NBCs.

Table I
Retention Times and Heart-Cut Times of NBCs

Analyte	DB-5MS RT (min)	Cut start (min)	Cut end (min)	HP-INNO AX RT (min)
Nitrobenzene	13.368	13.20	13.55	26.228
2-Nitrotoluene	23.559	23.40	23.80	38.547
3-Nitrotoluene	26.124	25.90	26.30	37.899
4-Nitrotoluene	21.810	21.66	22.06	35.200
4-Nitro-*o*-xylene	33.307	33.10	33.45	45.295
4-Nitro-*m*-xylene	32.328	32.15	32.50	46.634
4-Ntrocumene	35.064	34.90	35.25	44.998

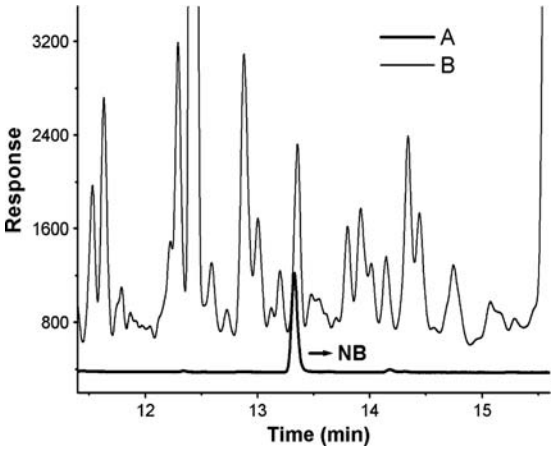

Figure 3. Overlay of first dimension chromatograms for (A) NB standard and (B) cigarette smoke sample.

Retention times on the DB-5MS column and cut times for individual NBCs were determined by injecting the standard with the fluidic switch set in the off position during the entire run. Table I listed the retention times of each NBC on the DB-5MS column, the cut start and stop times and the retention times of each peak cut to the HP-INNOWAX column.

Evaluation of heart-cutting chromatographic performance

Because the clean-up procedure was much simpler than the Hoffmann's method, all seven NBCs had heavy interferents or overlapped by other peaks on the first column. By following the optimized operational conditions detailed previously, the existing co-elution in the first dimension could be further resolved by transferring the selected cut to the 2D system. Nitrobenzene is used as an example to illustrate the advantages of heart-cutting 2D-GC.

As shown in Figure 3, many NBCs may be found in mainstream cigarette smoke by comparing the retention times of standard and smoke sample. However, when the suspected peak of nitrobenzene was cut to the second column, a series of peaks appeared in the range of 18–34 min after being separated by the 2D column (Figure 4). Moreover, there remained a large overlap in the 2D separation, but a peak for nitrobenzene from complex smoke matrix was clearly visible (Figure 4B). Figures 2 and 3B show that the suspected peak in the nitrobenzene cutting region in the 1D chromatogram primarily

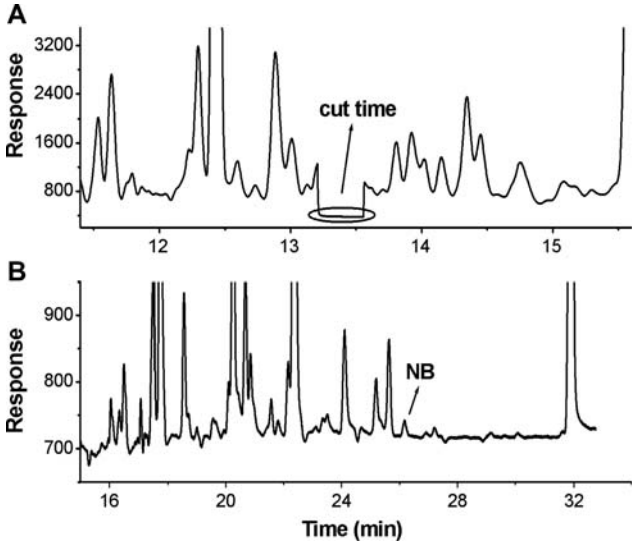

Figure 4. Chromatograms for (A) first column separation of cigarette smoke extract and (B) second column separation of NB heart-cut region from 13.20 to 13.55 min.

consisted of other interferents, and nitrobenzene accounted for only a very small fraction. The large number of peaks found in the 2D separation of complex smoke sample indicates that the resolution offered by one single column is insufficient and can easily lead to false-positive results. Compared to 1D-GC, the heart-cutting 2D-GC had not only better separating capacity, but also more reliable qualitative capacity. Figure 5 shows that there was nitrobenzene in mainstream cigarette smoke. After the 2D chromatographic separation, the background interference was greatly reduced, nitrobenzene was separated from interferences completely and the baseline was steady, which fully satisfied the quantitative requirements.

Method validation

Linearity and limit of detection

All nitrobenzenes examined were quantitatively analyzed under the GC conditions described in the "Experiment" section. The calibration curve of each compound, based on the peak area ratio of the target and the internal standard, showed good linearity in the range shown in Table II. Because 4-nitrotoluene was not detected in cigarette smoke (Figure 6), the other NBCs were quantified using 4-nitrotoluene as internal standard. The calibration curve and LOD of 4-nitrotoluene were measured by external standard calibration. Correlation coefficients (R^2) ranged between 0.9992 and 1. The limits of detection (LOD) were determined by analyzing the lowest level standard at least 10 times as an unknown over several days. The LOD (ng/mL) was then calculated as 3 times the standard deviation of these determinations. The method detection limit (ng/cig) of each NBC for both the ISO and HCI regimens are also shown in Table II.

Recovery and repeatability

Recovery was estimated by using fortified samples of cigarette smoke. These were prepared by adding a known amount of

Table II
Calibration Curve and LODs

Analyte	Calibration curve	Linear range (ng/ml)	R^2	LOD (ng/mL)	ISO MDL (ng/cig)	HCI MDL (ng/cig)
Nitrobenzene	Y = 2.6758X + 0.0129	5–300	0.9992	1.28	0.026	0.104
2-Nitrotoluene	Y = 0.8605X + 0.0084	25–3000	0.9999	2.07	0.041	0.164
3-Nitrotoluene	Y = 1.2683X − 0.0039	12.5–1000	1.0000	2.91	0.058	0.232
4-Nitrotoluene[a]	Y = 201.13X + 0.0231	12.5–500	0.9993	3.58	0.072	0.288
4-Nitro-*o*-xylene	Y = 0.6050X − 0.001	12.5–1000	0.9997	9.83	0.197	0.788
4-Nitro-*m*-xylene	Y = 0.3219X + 0.014	25–3000	0.9995	5.05	0.101	0.404
4-Ntrocumene	Y = 1.2333X + 0.0128	5–300	0.9992	3.15	0.063	0.252

[a]Because 4-nitrotoluene was not detected in cigarette smoke (Figure 6), the other NBCs were quantified using 4-nitrotoluene as internal standard. The calibration curve and LOD of 4-nitrotoluene were measured by external standard calibration.

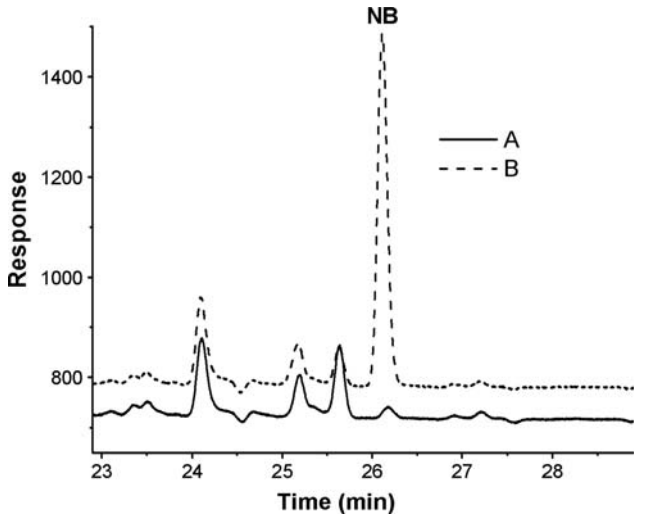

Figure 5. Magnified overlay of 2D chromatogram of NB heart-cut region from 13.20 to 13.55 min for (A) cigarette smoke sample and (B) fortified cigarette smoke sample with NB.

Table III
Recoveries ($n = 3$, %) at Three Spike Concentrations

Analyte	Added level (ng/cig)			Recoveries (%)		
Nitrobenzene	0.5	2	8	88.7	90.5	85.6
2-Nitrotoluene	12.5	50	200	104.9	104.4	111.1
4-Nitro-*m*-xylene	12.5	50	200	102.2	99.2	103.3
4-Ntrocumene	1.25	5	20	96.8	101.5	92.9

each NBC standard to the CFPs that had collected cigarette smoke according to the HCI regimen. For this recovery experiment, four groups of CFPs were prepared, three of which were spiked, in replicate, with a known amount of each target compound at three separate concentrations. All the spiked and unspiked CFPs were extracted, prepared and analyzed as described previously to determine the recovery of the spike. Data for these experiments are presented in Table III.

The relative standard deviation (RSD), a measure of repeatability, was evaluated by analyzing six replicates of 3R4F smoke extract. The RSDs were 6.82% for nitrobenzene, 2.79% for 2-nitrotoluene, 1.24% for 4-nitro-*m*-xylene and 12.78% for 4-ntrocumene, respectively.

Three NBCs, including 4-nitro-o-xylene, 3-nitrotoluene and 4-nitrotoluene, were not detected in mainstream cigarette smoke, so only the values of recovery and RSD for nitrobenzene, 2-nitrotoluene, 4-nitro-*m*-xylene and 4-ntrocumene were given in the experiment.

Table IV
Total Particulate Matter and NBCs Yields of Six Kinds of Cigarettes

Cigarette[a]	Regimen	TPM (mg/cig)	NBC yields (ng/cig)			
			Nitrobenzene	2-Nitrotoluene	4-Nitro-*m*-xylene	4-Ntrocumene
A	ISO	10.0	0.66	17.4	32.1	2.72
	HCI	39.6	2.22	69.8	98.0	12.3
B	ISO	2.56	0.078	Not detected	3.46	0.37
	HCI	23.5	0.98	26.2	64.7	5.03
C	ISO	14.6	1.66	40.2	38.0	2.10
	HCI	39.2	3.24	101.1	84.7	5.80
D	ISO	10.2	1.62	39.9	45.0	10.7
	HCI	34.6	4.80	155.8	160.4	38.8
E	ISO	9.2	0.69	22.3	22.6	1.73
	HCI	32.8	1.49	69.6	64.7	3.87
F	ISO	16.6	1.46	47.2	48.2	4.27
	HCI	34.9	1.98	85.3	94.4	6.68

[a]A, Kentucky reference cigarette, 3R4F; B, Kentucky reference cigarette, 1R5F; C, ZTRI reference cigarette, flue-cured type; D, ZTRI reference cigarette, blended type; E, F: Chinese commercial cigarettes

Application to real samples

Six kinds of Chinese and international cigarette brands were tested in the experiment, including two kinds of Kentucky blended reference cigarettes (3R4F, 1R5F), two kinds of Zhengzhou Tobacco Institute (ZTRI) reference cigarettes for hazard assessment (blended reference cigarette and flue-cured reference cigarette) and two brands of Chinese commercial cigarettes. All cigarettes were smoked according to two previously described smoking regimens (ISO3308 and HCI), and their smoke condensates were extracted, cleaned up and determined using the previously described method. The results are presented in Table IV. Although some suspicious peaks can be observed on the first column for all seven nitrobenzenes detected by Hoffmann in nonfiltered blended cigarette smoke, only nitrobenzene, 2-nitrotoluene, 4-nitro-*m*-xylene and 4-ntrocumene were found on the second column. 4-Nitro-*o*-xylene, 3-nitrotoluene and 4-nitrotoluene were not detected in the experiment because these compounds are not found in the modern cigarette smoke or because the amount of 4-nitro-*o*-xylene, 3-nitrotoluene and 4-nitrotoluene in the modern cigarette smoke is less than the LOD of this method.

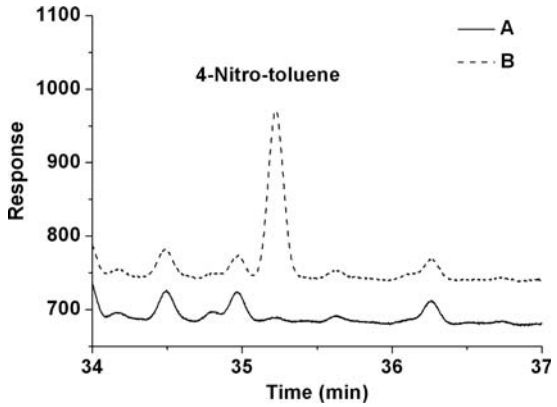

Figure 6. Magnified overlay of 2D chromatogram of 4-nitrotoluene heart-cut region from 21.66 to 22.06 min for (A) cigarette smoke sample and (B) fortified cigarette smoke sample with 4-nitrotoluene (as internal standard).

In addition, the values of the six cigarettes with the two smoking regimens were compared, and NBC yields of the same cigarette with the CHI regimen were approximately 2–20 times higher than those with the ISO3308 regimen, as shown in Table IV.

Conclusions

An effective method of determining nitrobenzenes in mainstream cigarette smoke was developed by a combination of SPE and heart-cutting 2D-GC. Compared with 1D-GC, the heart-cutting 2D-GC has stronger separation capacity, which leads to more accurate qualitative and quantitative results for NBCs. In addition, compared with the previous method determining NBCs in cigarette smoke, the current method simplified the pretreatment of smoke samples, supplied good repeatability and improved sensitivity and resolving power. Thus, it was more suitable for the analysis of NBCs in cigarette smoke, which provides a reference for the study on relationships among NBC level, smoking and smokers' health in the near future.

Acknowledgments

The authors gratefully acknowledge for the financial support of the National Nature Science Foundation of China (No. 20705040, 20875083, 21077095), the Scientific Foundation of the State Tobacco Monopoly Administration of China, and the Innovation Scientists & Technicians Troop Construction Projects of Zhengzhou City (10LJRC192). They also thank Dr. Julie Rimes for assistance with editing the text.

References

1. Rickert, D.E.; Toxicity of nitroaromatic compounds; Hemisphere Publishing, Washington, DC, (1985).
2. Smith, C.J., Perfetti, T.A., Rumple, M.A.; "IARC GROUP 2B carcinogens" report in cigarette mainstream smoke; *Food and Chemical Toxicology*, (2001); 39: 183–205.
3. International Agency for Research on Cancer; Nitrobenzene, printing processes and printing inks carbon black and some nitro compounds. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans, Lyon, France; (1996); pp. 381–408.
4. International Agency for Research on Cancer; Tobacco smoke and involuntary smoking. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Lyon, France; (2004); pp. 83.
5. Hoffmann, D., Rathkamp, G.; Quantitative determination of nitrobenzenes in cigarette smoke; *Analytical Chemistry*, (1970); 42: 1643–1647.
6. Hoffmann, D., Hoffmann, I., El-Bayoumy, K.; The less harmful cigarette: A controversial issue. A tribute to Ernst L. Wynder; *Chemical Research in Toxicology*, (2001); 14: 767–790.
7. Smith, C.J., Perfetti, T.A., Rumple, M.A., Rodgman, A., Doolittle, D.J.; "IARC Group 2B carcinogens" reported in cigarette mainstream smoke; *Food and Chemical Toxicology*, (2001); 39:283–205.
8. Hoffmann, D., Hoffmann, I.; The changing cigarette: Chemical studies and bioassays; *Smoking and Tobacco Control Monograph*, (2001); 13: 159–192.
9. Hecht, S.S.; Cigarette smoking: Cancer risks, carcinogens and mechanisms; *Langenbeck's Archives of Surgery*, (2006); 391: 603–613.
10. Schomburg, G.; Two-dimensional gas chromatography: Principles, instrument, methods; *Journal of Chromatography A*, (1995); 703: 309–325.
11. Deans, D.R.; Use of heart cutting in gas chromatography: A review; *Journal of Chromatography*, (1981); 203: 19–28.
12. Gordon, B.M., Rix, C.E., Borgerding, M.F.; Comparison of state of the art column switching techniques in high resolution gas chromatography; *Journal of Chromatographic Science*, (1985); 23: 1–10.
13. McCurry, J.D., Quimby, B.D.; Two-dimensional gas chromatography analysis of components in fuel and fuel additives using a simplified heart-cutting GC system; *Journal of Chromatographic Science*, (2003); 41: 524–527.
14. Gordon, B.M., Uhrig, M.S., Borgerding, M.F., Chung, H.L., Coleman, W.M., Elder, J.F.; Analysis of flue-cured tobacco essential oil by hyphenated analytical techniques; *Journal of Chromatographic Science*, (1988); 26: 174–179.
15. Muñoz-Arnanz, J., Bosch, C., Fernandez, P., Grimalt, J.O., Jimeñez, B.; Optimization of a heart-cutting multidimensional gas chromatography-based method for the assessment of enantiomeric fractions of *op*'-DDT in environmental samples; *Journal of Chromatography A*, (2009); 1216: 6141–6145.
16. Dunn, M., Shellie, R., Morrison, P., Marriott, P.; Rapid sequential heart-cut multidimensional gas chromatographic analysis; *Journal of Chromatography A*, (2004); 1056: 163–169.
17. Striebich, R.C., Rubey, W.A., Klosterman, J.R.; Trace-level measurement of complex combustion effluents and residues using multidimensional gas chromatography–mass spectrometry (MDGC–MS); *Waste Management*, (2002); 22: 413–420.
18. Bertsch, W.; Two-dimensional gas chromatography. Concepts, instrumentation, and applications—Part 1: Fundamentals, conventional two-dimensional gas chromatography, selected applications; *Journal of High Resolution Chromatography*, (1999); 22: 647–665.
19. Agilent G2855B Deans Switching System; Agilent Technologies Manual, Part Number: G2855–90120, 4th edition, (2008).