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Rapid Gas Chromatographic Analysis of Drugs of Forensic Interest

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Abstract

High-speed gas chromatographic (GC) screening for drugs of forensic relevance is performed using a commercial Flash™ GC instrument in which the chromatographic column is resistively heated at rates of up to 30°C/s. Temperature programming conditions are varied in an experiment designed to evaluate trade-offs between resolution and analysis time for a mixture of 19 drugs of abuse. All 19 components can be separated with excellent resolution in 90 s. Specific analytes can be analyzed even faster; for example, amphetamine analysis is completed in less than 20 s. Case studies of confiscated street drugs containing amphetamine, cocaine, and heroin are analyzed to evaluate the retention time repeatability. Ten replicate injections over a 2-day period for 3 different drug samples achieved retention time relative standard deviations in the range of 0.48 to 0.81%.

Introduction

Methods employing fast capillary gas chromatography (GC) have variously focused on narrow-bore columns, increased velocities, and rapid temperature programming to decrease separation times up to 2 orders of magnitude in comparison with conventional GC (1). However, classic trade-offs exist: fast temperature programming decreases the analysis time, but the resolution may be compromised. The design of chromatographic instruments for fast GC must maintain a narrow peak width throughout the system from injector to detector to produce good resolution (2,3).

The use of narrow-diameter capillary columns produces both higher efficiency (sharper peaks) and a flatter van Deemter curve at higher flow rates. This means that high flow rates can be employed without degrading efficiency. The introduction of a smaller internal diameter column was among the first attempts at rapid GC. Hyver and Phillips (4) and Ke et al.(5) demonstrated reduced analysis times using narrow capillary columns of 0.25- to 0.1-mm i.d. Lee et al. (6) were apparently the first to suggest resistive heating of the column to increase temperature programming rates and speed up analyses. Hail and Yost (7) developed a GC inlet probe for mass spectrometry (MS) using the direct resistive heating of aluminum-clad columns. Jain and Phillips (8) coated capillary columns with a thin conductive film for resistive heating to achieve analysis times measured in seconds. The uneven heating, compromised mechanical stability, and limited column life for such coated columns prompted Ehrmann et al. (9) to use a metal tube as the heating element combined with a sensor wire to measure the resistance (and thus the temperature) of the column. Improved methods to achieve fast temperature programming by resistive heating have resulted in applications such as high-speed air monitoring and screening for organic compounds (10,11). The commercially available Flash GC instrument (Thermedics, Chelmsford, MA) used in the present research achieves rapid analyses by direct resistive heating of the chromatographic column at rates up to 30°C/s without having to deal with the thermal mass of a column oven compartment.

In this article, the application of fast GC to rapid forensic screening for drugs of abuse is described. Because forensic laboratories and courts have become swamped with drug cases in recent years, the need to decrease analysis time and to improve case turnaround has grown. Conventional GC screening for a wide range of drugs is often considered impractical because of the lengthy analysis times required. The ability to perform screening for all major drugs of abuse in just a few minutes is a

Table I. Factor Definitions and Coded Levels for 2-Factor Central Composite Design

Factor	Coded factor levels				
	–1.41	–1	0	+1	+1.41
x_1, Time (s)	46	50	60	70	74
x_2, Temperature (°C)	201	205	215	225	229

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Table II. 2-Factor Central Composite Experimental Design and Measured Responses

Experiment	Run order	Time (coded units)	Temperature (coded units)	Retention time of last peak (s)	Resolution of methadone and methaqualone
1	7	1.00	–1.00	90.00	0.9793
2	10	0.00	–1.41	98.76	0.9385
3	5	1.00	–1.00	102.58	0.8977
4	3	–1.41	0.00	82.06	0.9793
5	12	–1.00	0.00	85.02	0.9181
6	2	0.00	0.00	91.88	0.9181
7	1	0.00	0.00	91.52	0.8365
8	11	0.00	0.00	91.24	0.8977
9	6	1.00	0.00	98.10	0.8365
10	9	1.41	0.00	101.10	0.8569
11	4	–1.00	1.00	78.68	0.7141
12	8	0.00	1.41	83.76	0.7957
13	13	1.00	1.00	93.42	0.7753

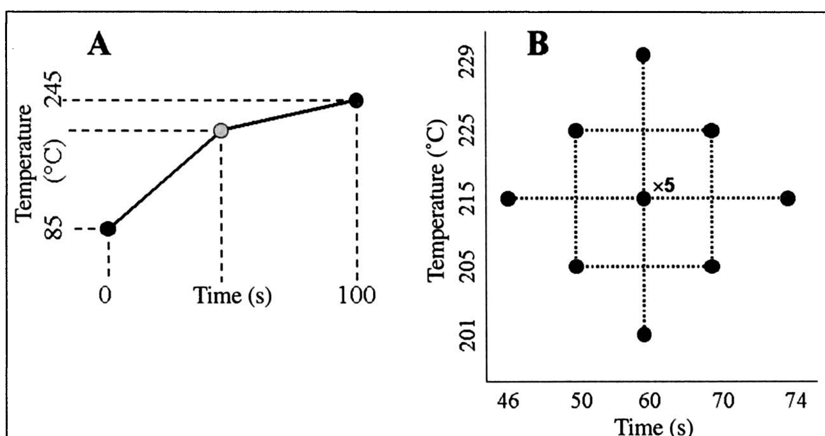

Figure 1. Two-ramp temperature program (A) starting at 85°C, ramped to an intermediate temperature (factor x_2) at a specified time (factor x_1), and then ramped to a final temperature of 245°C at 100 s. Two-factor central composite design (B) varying the location of the intermediate point in the temperature program with a total of 13 experiments, including 5 replicates of the center point.

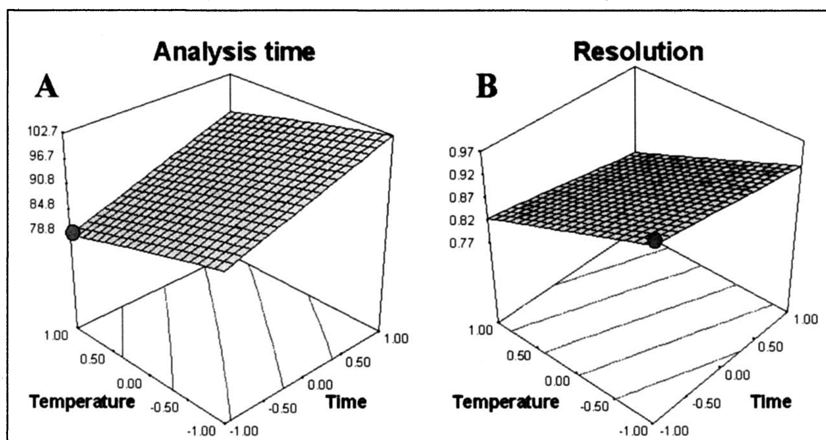

Figure 2. Fitted models for analysis time (A) and resolution responses (B). Dots mark the conditions of fastest analysis time and best resolution within the factor ranges shown. Coded factor levels are defined in Table I.

true incentive for the increased use of fast GC techniques for routine screening.

Experimental

Samples

Samples included a 10-ng/µL Toxiclean drug mixture (Alltech, Chicago, IL) containing amphetamine, methamphetamine, butabarbital, amobarbital, meperidine, pentobarbital, secobarbital, glutethimide, phencyclidine, methaqualone, methadone, cocaine, amitriptyline, imipramine, doxepin, desipramine, pentazocine, codeine, and oxycodone. Three case studies of forensic drug samples were obtained from the South Carolina Law Enforcement Division (SLED, Columbia, SC).

Chromatographic instrumentation

Analyses were performed on a Flash GC equipped with a flame ionization detector (Thermedics Detection, Chelmsford, MA). Separations were performed using a 6-m × 0.32-mm RTX-1 (Restek, Bellefonte, PA) fused-silica capillary column with a 0.1-µm film thickness. Approximately 1 µL of sample was injected into the injection port. Injection was performed in the split mode with a split vent flow of 70 mL/smin. The injector temperature was set at 250°C, the main oven heater was 300°C, and the detector temperature was 325°C. Helium was used as the carrier gas at a flow rate of 4.47 mL/min.

Results and Discussion

Resolution and analysis time trade-off

Designed experiments were employed to investigate the dependence of resolution and analysis time on experimental factors that determined the column temperature programming conditions. Two experimental factors (x_1, time; x_2, temperature; Table I) defining the intermediate point in a 2-ramp temperature program (Figure 1A) were varied in a 13-experiment central composite experimental design (Figure 1B and Table II) (12). The Toxiclean drug mixture was analyzed by the Flash GC under each set of conditions with experiments conducted in a random order.

Two performance measures were evaluated at each set of experimental conditions. The retention time of the last eluting peak was taken as a measure of chromatographic analysis time; the

resolution of two closely eluting peaks (methadone and methaqualone) was selected as a measure of resolution. These responses were fitted to full second-order models containing intercept, first-order, and second-order parameters for both factors in addition to a 2-factor interaction parameter. The fitted analysis time model produced a coefficient of determination (R^2) of 0.9992, and lack of fit was not significant at the 95% level of confidence (indicating that there is no reason to doubt the adequacy of the model). Linear trends in both factors and the 2-factor interaction were significantly different from zero at the 95% level of confidence. The fitted resolution model exhibited an R^2 value of only 0.7811; lack of fit was also not significant at the 95% level of confidence, and the linear trend in temperature was significantly different from zero at the 95% level of confidence. For the purpose of further investigation and discussion, the region of interest was limited to ± 1 coded units in each factor from the center of the design; plots of the 2 fitted models are displayed in Figure 2.

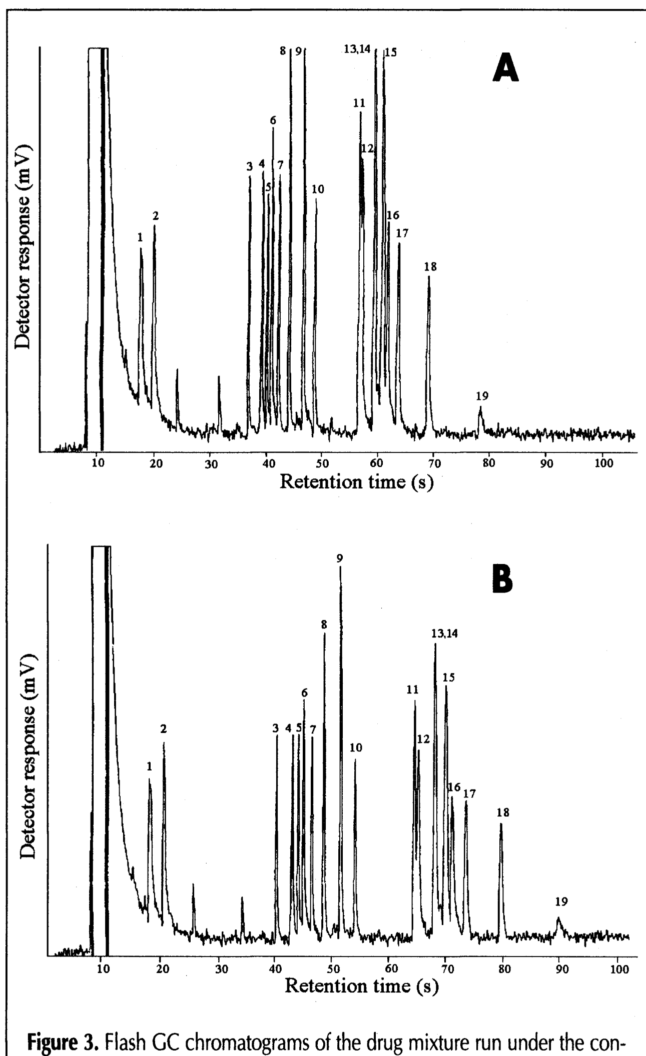

Figure 3. Flash GC chromatograms of the drug mixture run under the conditions of fastest analysis time (A) and best resolution of the methadone/methaqualone pair (B). Temperature program conditions are listed in Table III. Peak identification: 1, amphetamine; 2, methamphetamine; 3, butabarbital; 4, amobarbital; 5, penthidine; 6, pentobarbital; 7, secobarbital; 8, glutethimide; 9, PCP; 10, phenobarbital; 11, methadone; 12, methaqualone; 13, amitryptaline; 14, cocaine; 15, imipramine; 16, desipramine; 17, pentazocine; 18, codeine; and 19, oxycodone.

Table III. Flash GC Temperature Programs for Fastest Analysis Time and Best Resolution

Fastest analysis time		Best resolution of methadone/methaqualone pair	
Time (s)	Temperature (°C)	Time (s)	Temperature (°C)
0	85	0	85
50	225	60	215
100	245	100	245

Chromatograms produced under the conditions of fastest analysis time and best resolution with these constraints are shown in Figure 3. The response surfaces and chromatograms taken together illustrate the classic trade-off between analysis time and resolution. The two goals cannot be simultaneously achieved at the same experimental conditions (Table III). Because the present objective was rapid drug screening, and because samples were not expected to contain every component found in the test mixture, the conditions of fastest analysis time were chosen for all subsequent runs.

Case studies

The results of 3 case studies are presented here to illustrate the validity and applicability of fast GC for rapid drug screening method in the forensic laboratory. Case studies were taken from forensic drug samples submitted to the Drug Identification Department of SLED. These samples were analyzed using the conditions of fastest run time determined from the experimental design.

Representative chromatograms of case study samples containing amphetamine (case 1), cocaine (case 2), and heroin (case 3) are shown in Figures 4–6. The resolution and peak intensity for each of these target analytes was sufficient to determine each drug's presence in the sample by matching retention times to a standard sample. Each case study was also analyzed using conventional GC–MS, and major component identities were confirmed by comparing mass spectra to library spectra. Additional peaks in each chromatogram (not identified) are caused by impurities in the preparation of these confiscated drugs.

Table IV summarizes the reproducibility and repeatability of retention times for the 3 case study samples. Reproducibility was investigated by calculating the percent relative standard deviation (%RSD) of each analyte's retention time from each set of

Table IV. Retention Time Reproducibility and Repeatability for Selected Drugs of Abuse

Drug	% RSD Runs 1–5	Runs 6–10	Runs 1–10
Amphetamine	0.608	0.756	0.652
Cocaine	0.806	0.330	0.761
Heroin	0.550	0.432	0.475

5 consecutive runs. The average %RSD for the retention times over all the analytes for the 2 sets of data was 0.580%. Repeatability was investigated by calculating the %RSD of the retention times over the 10 runs combined from both days; the average %RSD over the 2-day period for all samples was 0.629%.

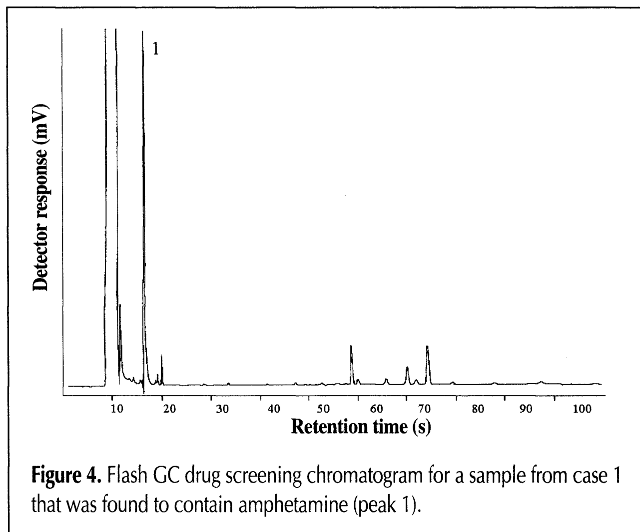

Figure 4. Flash GC drug screening chromatogram for a sample from case 1 that was found to contain amphetamine (peak 1).

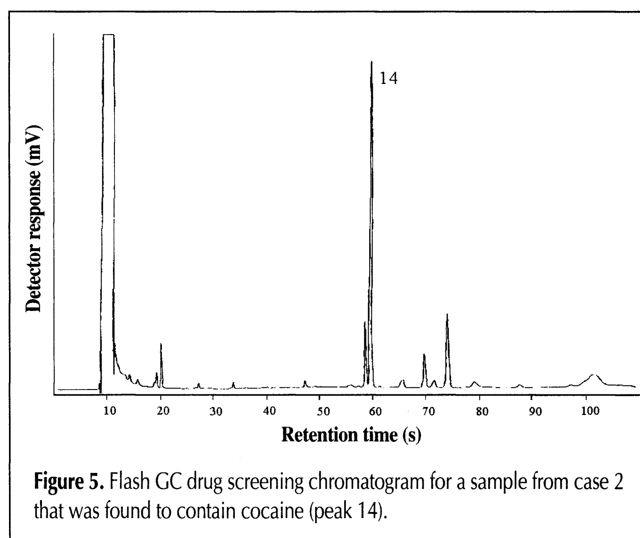

Figure 5. Flash GC drug screening chromatogram for a sample from case 2 that was found to contain cocaine (peak 14).

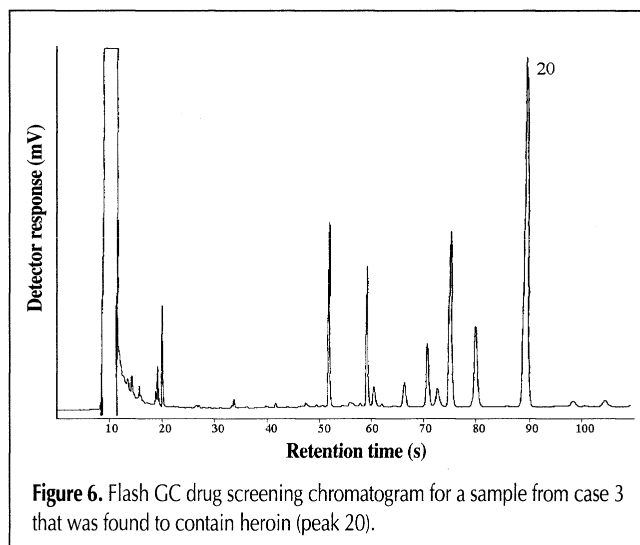

Figure 6. Flash GC drug screening chromatogram for a sample from case 3 that was found to contain heroin (peak 20).

These results demonstrate excellent retention time reproducibility with little day-to-day variation in retention times (an essential requirement if retention times are to be used by themselves for peak identification in routine screening by such fast GC methods). All injections were manually performed using a syringe; better reproducibility might be expected with an autosampler.

Conclusion

Fast GC techniques offer rapid routine forensic screening for drugs of abuse. The analysis of a test mixture of 19 different drugs could be completed in 80 s using Flash GC in comparison with up to 15–20 min using conventional GC systems (0.25-mm i.d. columns, 0.25-µm stationary phase film thickness). In the case studies described here, GC runs for identifying specific target analytes could be completed even faster: less than 20 s for amphetamine, 60 s for cocaine, and 90 s for heroin. The excellent retention time reproducibility and minimal day-to-day variability supports the use of retention time matching for rapid component identification. The application of fast GC, coupled with the development of rapid extraction methods as described in a separate publication (13), should have a tremendous impact in routine drug screening, forensic toxicology, and clinical applications.

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