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SIMULTANEOUS GAS CHROMATOGRAPHIC ANALYSIS OF DRUGS OF ABUSE ON TWO FUSED-SILICA COLUMNS OF DIFFERENT POLARITIES

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SUMMARY

A convenient and rapid gas chromatographic method for analysis of drugs of abuse has been developed. By installing two differently coated columns in a common split–splitless injector and connecting the column ends to a nitrogen–phosphorus detector and a flame ionization detector, considerable and accurate chromatographic information could be obtained from a single run. Quantitative analyses of amphetamine and some other drugs have been performed with the same instrumental set-up. Calibration graphs were evaluated and showed linearity in the concentration range chosen (0.5–10 mg/ml). By using an auto-sampler in combination with a BASIC program, both types of analyses can be performed routinely.

INTRODUCTION

Capillary gas chromatography (GC) has so far received comparatively little attention in forensic drug analysis. Some of the problems associated with the successful use of capillary columns have been due to unwanted surface activity, poor wettability and relatively low thermostability[1]. The rapid development in column technology during recent years will most certainly change this situation. Refined methods of coating and deactivation of glass capillaries have made possible the preparation of columns of high quality and efficiency. Recently, Blomberg and co-workers[2,3] demonstrated the usefulness of such columns for the separation of underivatized drugs on four different types of liquid phases. Central nervous system stimulants in urine were studied by Kinberger *et al.*[4] who used SP-2100 as liquid phase, and Pettitt[5] reported on a screening method for drugs of abuse on a SP-2250 column. A few other non-polar phases suitable for toxicological screening were tested by Schepers *et al.*[6] and Plotczyk[7].

The immobilization of liquid phases has further improved the versatility of capillary GC[8–11]. Such columns generally show increased thermostability, have little bleeding and therefore larger practical temperature ranges. Some of the problems

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frequently encountered in toxicological work, *e.g.*, deterioration of the thin liquid phase due to biological impurities and water, should be easier to overcome.

The introduction of flexible and less fragile fused-silica columns has provided a material suitable for routine analyses. A further advantage of fused silica is the possibility of inserting two capillaries in a common injection port[12,13]. With such an instrumental configuration, differently coated columns connected to two (or more) different detectors can be used and considerable information obtained in one chromatographic run. This paper describes a chromatographic method primarily used for rapid screening of illicit drug samples and other similar compounds of forensic interest.

EXPERIMENTAL

Apparatus

The analyses were performed using a Hewlett-Packard Model 5880A gas chromatograph equipped with BASIC programming capabilities, a split–splitless capillary inlet system operating in the split mode, a flame ionization detector (FID), a nitrogen–phosphorus detector (NPD), two terminals (printer-plotters) and an automatic sampler (HP 7672A). Two fused-silica columns (Scientific Glass Engineering, Victoria, Australia; 0.2 mm I.D.) were deactivated, statically coated and the stationary phases immobilized following the procedure described by Blomberg *et al.*[3]. One column (SE-54, 11 m, film thickness 0.26 μm) was connected to the FID and the other (OV-215, 10 m, film thickness 0.20 μm) to the NPD. A graphite ferrule (SGE Graphlok GFF/010) was used to form a leak-free seal on the injector side. Other conditions: carrier gas (helium) velocity, 40 cm/sec (splitting ratio 40:1); make-up gas (helium NPD) flow-rate, 30 ml/min; air gas flow-rate, 60 ml/min (NPD) and 400 ml/min (FID); hydrogen gas flow-rate, 3 ml/min (NPD) and 30 ml/min (FID); background current (NPD), 17 pA.

Chemicals and sample preparation

All drugs were of pharmaceutical grade and of different origins. Amphetamine sulphate (analytical grade) was supplied from ADA (Apotekarnas Droghandel, Stockholm, Sweden). N-Propylamphetamine was synthesized according to Moore[14] and then recrystallized twice from ethanol. Trioctylamine (Merck, 95%) was converted into the hydrochloride, which was purified by repeated recrystallization from diethyl ether–hexane (1:1). Drug standard solutions (1 and 10 mg/ml) and unknown sample solutions (10 mg/ml) were prepared by adding the solute(s) to an aqueous solution (1 ml) of propylamphetamine (5 mg/ml). Two drops of concentrated ammonia were then added and the mixture shaken with a solution (1 ml) of trioctylamine (2 mg/ml) in ethyl acetate. After filtering over $MgSO_4$, 1 μl was injected. The barbiturates (2 mg/ml) were added to an acidified (hydrochloric acid) aqueous solution (1 ml) containing dipropylbarbital and methylphenylbarbital (2 mg of each per ml) as internal standards and extracted with 1 ml of diethyl ether. After drying ($MgSO_4$), 1 μl was injected.

Calibration graph

A calibration graph for amphetamine in the concentration range 0.5–10 mg/ml

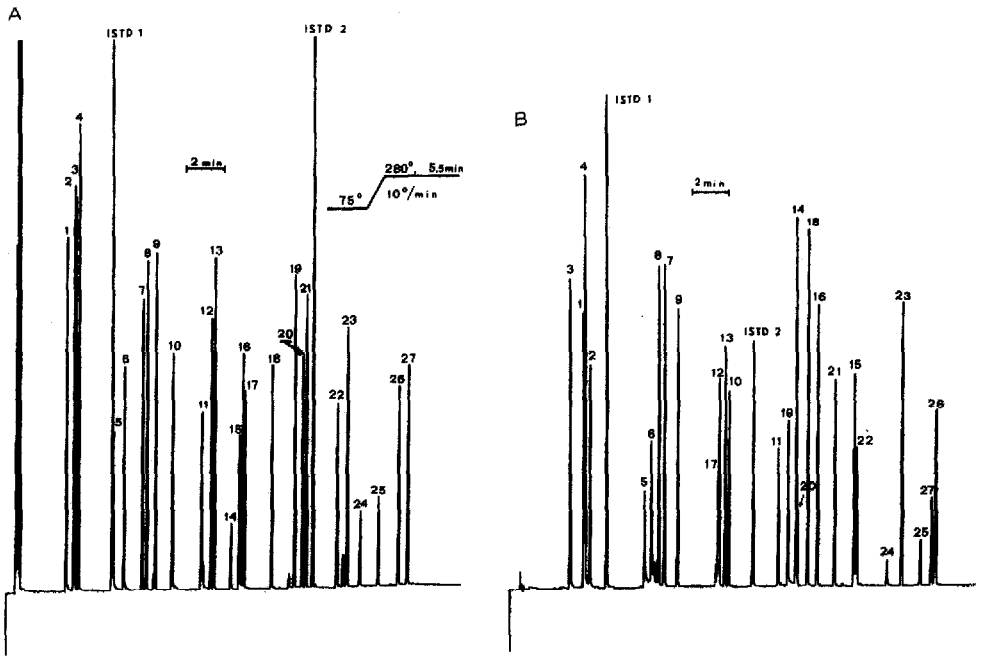

Fig. 1. Gas chromatograms of illicit drugs and similar compounds on fused silica. A, Column coated with immobilized SE-54 (FID, attenuation × 8); B, column coated with immobilized OV-215 (NPD, attenuation × 128). Substances: 1 = amphetamine; 2 = phentermine; 3 = propylhexedrine; 4 = methylamphetamine; 5 = norephedrine; 6 = ephedrine; 7 = phenmetrazine; 8 = phendimetrazine; 9 = amfepramone; 10 = benzocaine; 11 = phenacetine; 12 = methylphenidate; 13 = pethidine; 14 = caffeine; 15 = phenazone; 16 = lidocaine; 17 = phencyclidine; 18 = procaine; 19 = methadone; 20 = dextropropoxyphene; 21 = cocaine; 22 = codeine; 23 = diazepam; 24 = acetylmorphine; 25 = heroin; 26 = flurazepam; 27 = papaverine.

was constructed using propylamphetamine as internal standard (Fig. 4). Two runs were made for each sample. The test solutions were prepared as described above. Aliquots of 1 μl were injected.

BASIC programming

A BASIC program was written such that the relative retention times (RRTs) from both detector signals were calculated from two internal standards. The obtained values were then compared to stored RRT-windows empirically determined for different concentrations of individual substances. The names of the compounds identified in this way were then printed out in the chromatogram. For concentration measurements the standard software of the instrument was utilized, but both types of measurements (qualitative and quantitative) were monitored by the specially designed BASIC program.

RESULTS AND DISCUSSION

Qualitative analysis

A dual-column analysis of a standard mixture is illustrated in Fig. 1. Chroma-

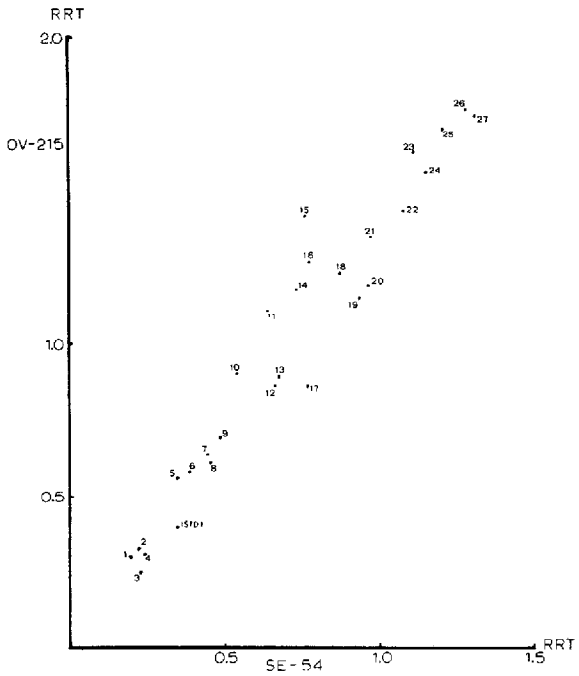

Fig. 2. Correlation of relative retention times of drugs and similar compounds on SE-54 and OV-215 columns. Numbers refer to substances in Fig. 1.

togram A was obtained on a SE-54 column connected to the FID and chromatogram B on a OV-215 column connected to the NPD. Both columns were prepared in the authors' laboratory and are of medium quality (height equivalent to a theoretical plate, HETP = 0.25 and 0.31 mm respectively; capacity ratio, k = 15, C_{14}). The resolution is, however, sufficient for the applications and enabled the use of rather short columns (10–11 m). The particular combination of stationary phases was chosen according to the desired properties of high temperature stability (large temperature ranges), sufficiently large difference in polarity for discrimination of a large number of compounds with gross structural variations (drugs, additives, adulterants, etc.) and the use of a suitable common temperature program. The usefulness of the column combination is easily seen in the chromatograms (Fig. 1). Most of the compounds on SE-54 appear in a different order of elution on OV-215.

For practical reasons and simplicity, relative retention times (RRTs) were used in this study. In the low temperature region, propylamphetamine (ISTD 1), and in the high temperature region trioctylamine (ISTD 2), were used as internal standards. The correlation of the RRT values (relative to ISTD 2) obtained with the two phases is shown in Fig. 2. Retention time reproducibility is very good with modern gas chromatographs (standard deviation usually less than 0.1 %) and this is further enhanced by using an automatic sampler. The limiting factor determining the width of the RRT-windows was therefore the practical concentration range chosen (1–10 mg/ml). Individual RRT-windows (two sets) were determined and the values stored in the programs for fully utilizing the capabilities of the method. In Fig. 2 all windows have

been given a typical value of 0.01 RRT unit. In practice, however, there are individual variations, both in shape and magnitude, but these are to small to be clearly visualized in the figure.

Although there is a clear correlation between the RRT values of the phases chosen (Fig. 2), the spread of the values is large compared to the size of the windows and therefore the risk of accidental overlap is small. This risk is, of course, further reduced by the FID–NPD combination of detectors. The validity of the system was tested during 6 months by recording mass spectra in parallel with GC runs of more than 2000 casework samples. In no case were false positives registered with the approximately 80 compounds encountered. It should also be pointed out that the GC analyses are used in combination with other analytical methods, high-performance thin-layer chromatography and UV spectroscopy, for final identification.

The splitting ratios and linearity of detector responses at different concentrations did not vary noticeably over the testing period (see below). A more detailed examination of these parameters in dual-column operation has recently been published[13]. For calibration purposes, however, the system was found to be sensitive to alterations. Thus, if the columns were taken out from the oven and then reinstalled and the carrier gas pressure had been altered, slight changes in the RRT values were observed after readjustment of the pressure. In such a case recalibration was found necessary. Part of this effect may be due to the coarseness of the manometers used (range 1–200 kPa).

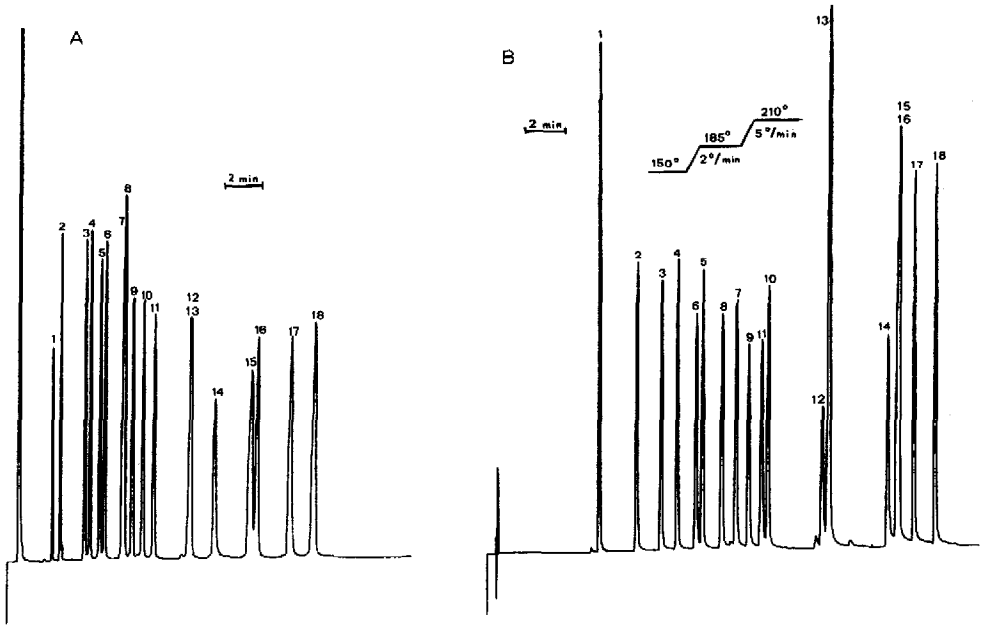

Fig. 3. Gas chromatograms of underivatized barbiturates on fused silica columns. A, SE-54 (FID, attenuation × 2); B, OV-215 (NPD, attenuation × 16). Substances: 1 = metarbital; 2 = barbital; 3 = allobarbital; 4 = aprobarbital; 5 = ISTD 1; 6 = butalbarbital; 7 = amobarbital; 8 = nealbarbital; 9 = pentobarbital; 10 = vinbarbital; 11 = secobarbital; 12 = brallobarbital; 13 = hexobarbital; 14 = ISTD 2; 15 = phenobarbital; 16 = cyclobarbital; 17 = alphenal; 18 = heptabarbital.

Long-term changes of column performance were regularly studied by injection of test mixtures. In order to increase durability and temperature stability, both phases were treated with dicumyl peroxide in the coating step[3]. After a test period of 6 months (about 2000 injections) the SE-54 phase was found to be remarkably stable with no measurable decrease in efficiency or capacity ratio. With the OV-215 phase, however, absolute retention times were found to change slowly with time (0.6 min in total). Since the efficiency of the column was unaltered this is an indication of phase stripping. The recommended maximum allowable temperature for untreated OV-215 (trifluoropropylmethyl silicone gum) is specified by the manufacturer to be 250–275°C, whereas 280°C was used in this study. It is, however, difficult to tell whether this phase stripping depends on chemical decomposition of the phase by pyrolysis or is a surface catalytic effect which induces decomposition. Further testing is therefore required properly to evaluate the durability of the columns.

Fig. 3 shows another application with the same instrumental set-up. Underivatized barbiturates were separated with very little tailing using a modified temperature program. Dipropylbarbital (ISTD 1) and methylphenylbarbital (ISTD 2) were used as internal standards and a calibration table was constructed using the standard software supplied with the instrument (HP 5880A, level 4).

Quantitative analysis

Quantitative analysis (internal standard method) by capillary GC using the split injection technique requires linearity of the splitting. If this is the case, quantitative results achieved by capillary columns are often superior to those obtained using packed columns.

Amphetamine is presently one of the most common drugs of abuse in Sweden. It was therefore desirable to combine the screening method with a quantitative method using the same auto-injector. With the instrumental set-up described above, amphetamine (and occasionally other drugs) were analyzed on the SE-54 column

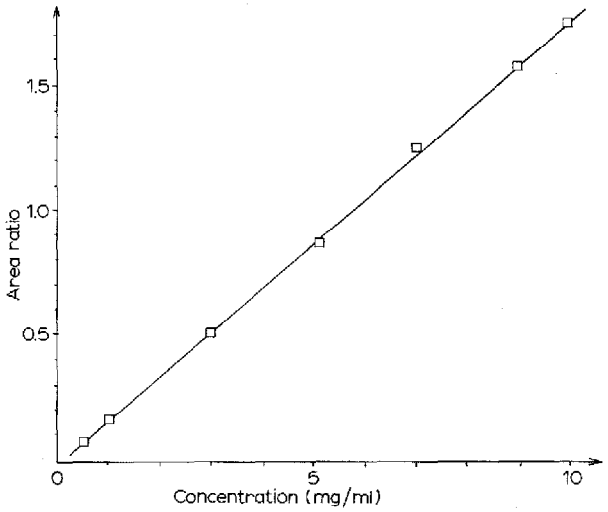

Fig. 4. Calibration graph of amphetamine in the concentration range 0.5–10 mg/ml. Details are given in the text.

connected to the FID using a shorter temperature program. In Fig. 4 the ratios between the peak areas of sample and internal standard (propylamphetamine) are plotted against known amounts of the compound in mg/ml. Splitting was found to be linear and accurate and reproducible results could be obtained. The error of the method was estimated to be ± 0.22 mg/ml if a 95% confidence interval was chosen*.

Linear calibration curves were also obtained with other drugs such as methylamphetamine, phenmetrazine, phendimetrazine and cocaine. For heroin, injected as the hydrochloride in methylene chloride solution, the calibration curve was not reproducible. The reason for this is not yet fully understood but it is known that heroin is unstable at high temperatures.

CONCLUSION

No practical problems are associated with the use of two capillary columns in the same injector and the results are comparable to those obtained from a single-column analysis. This makes the method particularly suitable for use in laboratories handling a large number of routine analyses.

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* The accuracy of the method within the calibrated range was estimated using an approximate 95% confidence interval for the predicted value of the concentration, x. The limits for x then become[15]

$$x \pm \frac{t_\alpha s}{b} \cdot \left(\frac{1}{m} + \frac{1}{n} + \frac{(y_0 - \bar{y})^2}{b^2 S_{xx}} \right)^{1/2}$$

where t_α is the value given by "Student's" t-distribution for a probability, α ($=0.025$), of lying outside the interval and $n - 2$ degrees of freedom, s is the standard deviation of the least-squares fit for the calibration, b is the slope, m is the number of separate determinations for each data point, n is the number of data points, y_0 is the observed peak area ratio, $\bar{y}$ is the mean value of peak area ratios and $S_{xx} = \sum_i x_i - 1/n(\sum_i x_i)^2$; summation is made over all x values. From the calibration of amphetamine ($n = 7$, $s = 0.016$, $b = 0.175$, $t_\alpha = 2.57$ for $n - 2$ degrees of freedom, $m = 2$ and $S_{xx} = 85.645$) the limits obtained were $x \pm 0.22$ mg/ml. A simpler, but less stringent, approximation of the error in the determination of x can be given as $\pm 2s/b$, using two standard deviations. Thus, for amphetamine the error obtained is ± 0.18 mg/ml. The accuracy of the method is given by an absolute error over the calibrated range. This means that the relative error is much greater in the lower concentration range. In order to compensate for this, larger sample amounts have to be used.

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