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Note

Glass capillary column gas chromatography of narcotic drugs after flash-heater trimethylsilylation

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Glass capillary column gas chromatography is widely used for the analysis of biologically important substances such as steroid hormones[1] and fatty acids[2]. However only a few papers have been published on drug analysis[3–5] in spite of the fact that improved specificity and sensitivity are obtained with capillary columns in comparison with packed columns. It is often necessary to derivatize the drugs before gas chromatography. Even good glass capillary columns often show undesirable adsorption characteristics and the problem becomes more serious with decreasing amounts of sample. One means of overcoming this problem is to convert polar drugs into non-polar derivatives. Derivatization may also increase the resolution and the difference in the retention times of a drug, and the drug derivative may give extra information during the identification of unknown substances. This is of importance in the analysis of narcotic drugs.

In earlier papers we have described the technique of on-column derivatization[6–9]. Packed columns were used in these studies and the silyl and acyl derivatives of the drugs were formed by simultaneous injection of the sample and the reagent into the column. The method was used successfully for qualitative and quantitative drug analyses using flame-ionization and electron-capture detection. It was the purpose of this investigation to show that derivatization of drugs in a heated capillary injector (flash-heater derivatization) can be used for resolving complex drug mixtures and also for quantitative drug analysis in the nanogram range.

Narcotic drugs were used as model substances and reagents suitable for forming trimethylsilyl derivatives are studied. The reproducibility of quantitative analysis and the minimal detectable amounts of codeine and morphine were evaluated and the results are compared with earlier findings.

MATERIALS AND METHODS

Reagents

Table I lists the compounds used. Methyl phenidate and phenmetrazine were supplied by Ciba-Geigy (Basle, Switzerland). Hexadecane and eicosane were purchased from Koch-Light (Colnbrook, Great Britain). Heroin was synthesized from morphine. The other drugs were of pharmacopoeial grade and supplied by Nors

Medisinaldepot (Oslo, Norway). Ampoules of 1 ml of N,O-bis(trimethylsilyl)-acetamide (BSA), N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-trimethylsilylimidazole (TMSIM), used as derivatization reagents, were purchased from Supelco (Bellefonte, Pa., U.S.A.). After opening the ampoules were kept as described earlier[9].

Analytical-reagent grade ethyl acetate and chloroform were obtained from E. Merck (Darmstadt, G.F.R.). Stock standard solutions of the compounds were prepared in ethyl acetate at concentrations of 5 mg/ml, except for morphine, the concentration of which in ethyl acetate was 1 mg/ml. These solutions were diluted to give a concentration of 250 μg/ml for gas chromatography.

Gas chromatography

A Fractovap 2300 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a flame-ionization detector (FID) and a capillary column splitless injector was used. The glass capillary column (20 m × 0.35 mm I.D.) (H. and J. Jaeggi, Trogen, Switzerland) was wall-coated with SE-30. The injection port temperature was 250° and the samples were injected at an oven temperature of 50° or 70°. The temperature was programmed at 5°/min up to 250°. Nitrogen was used at the carrier gas at an inlet pressure of 0.4 kp/cm^2, which gave a flow-rate of 1.4 ml/min through the column. The splitting ratio of the injector was 1:40 for the identification test and the sensitivity setting was 10 × 8.

Samples for the calibration graph were injected without splitting. The splitter was closed before the injection and re-opened to a splitting ratio of 1:40 30 sec after the injection. The sensitivity setting was 1 × 8 and a Spectra Physics Autolab Minigrator was connected to the gas chromatograph for peak area measurements.

Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) was carried out using a Varian Model 112 mass spectrometer (Varian-MAT, Bremen, G.F.R.) combined with a Varian Model 1400 gas chromatograph (Varian, Walnut Creek, Calif., U.S.A.). The glass capillary column (50 m × 0.28 mm I.D.) (LKB, Stockholm, Sweden) was wall-coated with SE-30.

Identification of narcotic drugs

A mixture of the compounds listed in Table I was prepared to give a concentration of 250 μg/ml in ethyl acetate for each. This solution was also diluted 1:10 and 1:100. A 1-μl volume of derivatization reagent was drawn into the syringe (Hamilton 701 N) followed by 1 μl of the test solution, and the mixture was injected into the gas chromatograph. About 50 mg of the illicit heroin samples were dissolved in 50 ml of chloroform, the solutions were centrifuged and 1 μl was injected into the gas chromatograph together with 1 μl of derivatization reagent.

Calibration graph

Calibration graphs for the concentration range 1–10 μg/ml in ethyl acetate were constructed for codeine and morphine using ethylmorphine as internal standard. The concentration of the internal standard was 10 μg/ml. A 1-μl volume of BSA was injected into the gas chromatograph together with 1 μl of the test solution. The

peak-area ratios (drug derivative to internal standard derivative) were plotted against the drug concentration. Five assays of each solution were carried out and the regression lines and the correlation coefficients were calculated.

Reproducibility of quantitative analysis after derivatization

A test solution containing 5 μg/ml of codeine and morphine and 10 μg/ml of internal standard was analysed as described above and the peak-area ratios were calculated. The mean and the relative standard deviations (RSDs) for ten analyses were calculated.

RESULTS AND DISCUSSION

Information relating to some of the drugs that form trimethylsilyl derivatives under the above conditions are given in Table I. The results obtained by injecting a mixture of fourteen different compounds together with BSA is shown in Fig. 1. The mixture diluted 1:10 to give a concentration of 25 μg/ml also gave peaks that permitted easy identification. When the mixture was diluted 1:100 to give a concentration of 2.5 μg/ml, the attenuation had to be set to a higher value, and impurities from BSA interfered with the early eluted drugs. With more purified reagents the compounds can also be easily identified in this concentration range.

TABLE I

COMPOUNDS EXAMINED AND THEIR ELUTION CHARACTERISTICS

Compound	*Functional group for derivatization*	*Compound derivatized*	*Temperature when compound was eluted (°C)*	*Temperature when derivative was eluted (°C)*
Amphetamine	Primary amine	Yes	91 (small, broad)	96
Ephedrine	Secondary amine Hydroxyl	Yes Yes	105	108
Phenmetrazine	Secondary amine	No	113	
Hexadecane	None	No	137	
Methyl phenidate	Secondary amine	No	148	
Pethidine	None	No	150	
Caffeine	None	No	163	
Eicosane	None	No	180	
Methadone	None	No	192	
Cocaine	None	No	198	
Codeine	Hydroxyl	Yes	216 (broad)	220
Ethylmorphine	Hydroxyl	Yes	219 (broad)	223
Morphine	Hydroxyl	Yes	—	227
Heroin	None	No	236	

The identity of the drug derivatives was checked by GC–MS. This investigation showed that N,N-bis(trimethylsilyl)amphetamine, N,O-bis(trimethylsilyl)ephedrine, O-trimethylsilylcodeine, O-trimethylsilylethylmorphine and O,O-bis(trimethylsilyl)morphine were formed. No underivatized drug could be detected, and it was concluded that the reaction was complete. The secondary cyclic amines in methyl phenidate and phenmetrazine were not derivatized, and no derivative formation was

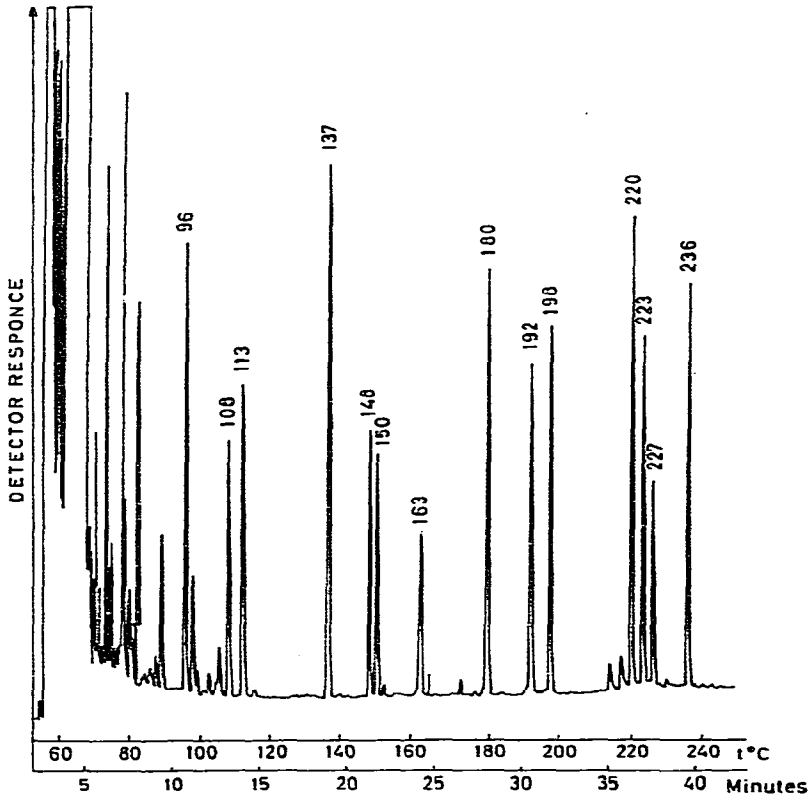

Fig. 1. Chromatogram obtained after flash-heater derivatization by injecting 1 μl of BSA together with 1 μl of an ethyl acetate solution containing 250 ng of each of the following components: 96 = amphetamine; 108 = ephedrine; 113 = phenmetrazine; 137 = hexadecane; 148 = methyl phenidate; 150 = pethidine; 163 = caffeine; 180 = eicosane; 192 = methadone; 198 = cocaine; 220 = codeine; 223 = ethylmorphine; 227 = morphine; 236 = heroin. Column temperature: 50° at start, programmed at 5°/min.

observed when the volume of BSA injected was increased. The lower reactivity of secondary amines has been described for several derivatization reagents[10].

The drugs were also dissolved in solvents such as chloroform, benzene and carbon disulphide and the reaction was found to be independent of the solvent. It is well known that silylation reactions with amine groups are markedly dependent on the solvent when performed off-column[10]. The same derivatives were also formed with the drug salts dissolved in chloroform.

BSTFA and TMSIM were also tried as derivatization reagents but only with BSTFA was the reaction complete. TMSIM, which is the preferred reagent for the silylation of all OH groups, will not derivatize primary and secondary aliphatic amines[10]. This reagent therefore cannot be used for the identification of amphetamine and ephedrine.

Fig. 2 shows a chromatogram relating to an illicit heroin sample that was extracted with chloroform and derivatized with BSA. From this chromatogram caffeine, morphine, monoacetylmorphine and heroin could be detected. The peak eluted at 275° was identified as strychnine. A GC–MS investigation was also carried

out to verify the results. The experimental aspects of the splitless sampling technique have recently been evaluated[11]. To avoid overloading of the column, it was necessary to inject the codeine–morphine samples without splitting at an oven temperature of 70° and to re-open the splitter 30 sec after the injection.

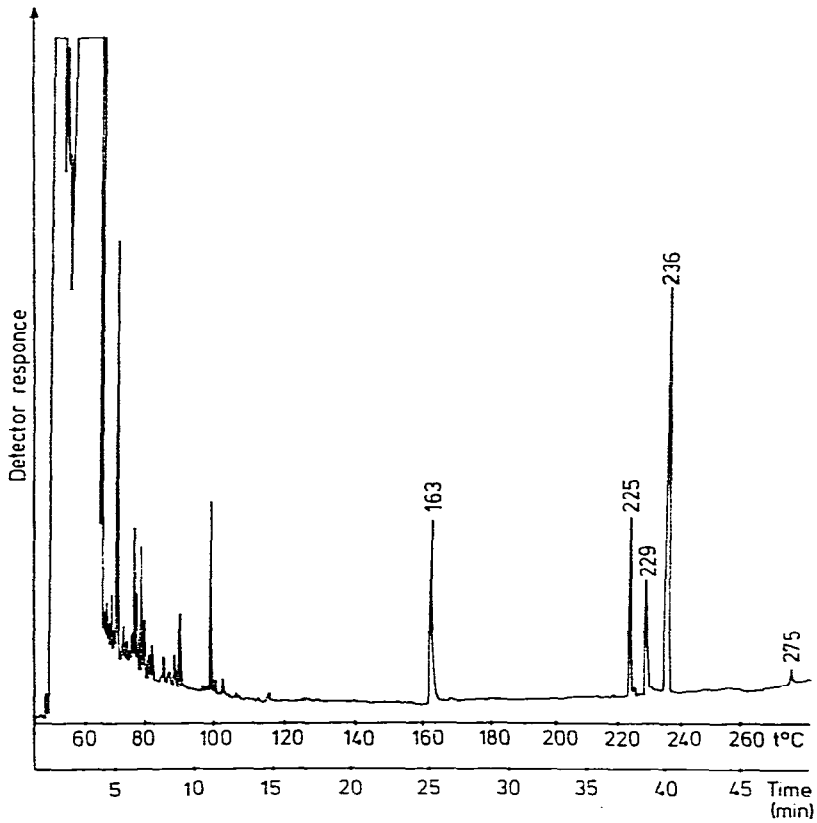

Fig. 2. Chromatogram obtained after flash-heater derivatization of a chloroform extract of an illicit heroin sample. A 1-μl volume of BSA was injected together with 1 μl of the sample. 163 = Caffeine; 225 = monoacetylmorphine; 229 = morphine; 236 = heroin; 275 = strychnine. Column temperature: 50° at start, programmed at 5°/min.

In order to check the linearity of the derivatization method in the temperature-programmed mode, calibration graphs were constructed for codeine and morphine. The calibration graphs were calculated according to the method of least squares, relating y (the peak-area ratio of the drug derivative to the internal standard derivative) to x (the concentration of the drug solution in micrograms per millilitre). The calibration graph for the concentration range 1–10 μg/ml was $y = 0.100x - 0.033$ with a correlation coefficient of 0.9991 for codeine and $y = 0.083x - 0.063$ with a correlation coefficient of 0.9963 for morphine.

The data obtained from the reproducibility test showed that at 5.0 μg/ml the RSD was 1.5% for codeine and 4.3% for morphine. These values are similar to those found earlier using packed columns for the analysis of codeine after on-column

derivatization with heptafluorobutyrylimidazole, heart-cutting and electron-capture detection[9].

Fig. 3 shows a chromatogram after flash-heater silylation of 10 ng of codeine, 10 ng of ethylmorphine and 10 ng of morphine. A blank run showed no interfering peaks with the codeine, ethylmorphine or morphine derivative. With this method the minimal detectable amount was about 500 pg for codeine and morphine. However, with the earlier described method with electron-capture detection smaller amounts could be detected.

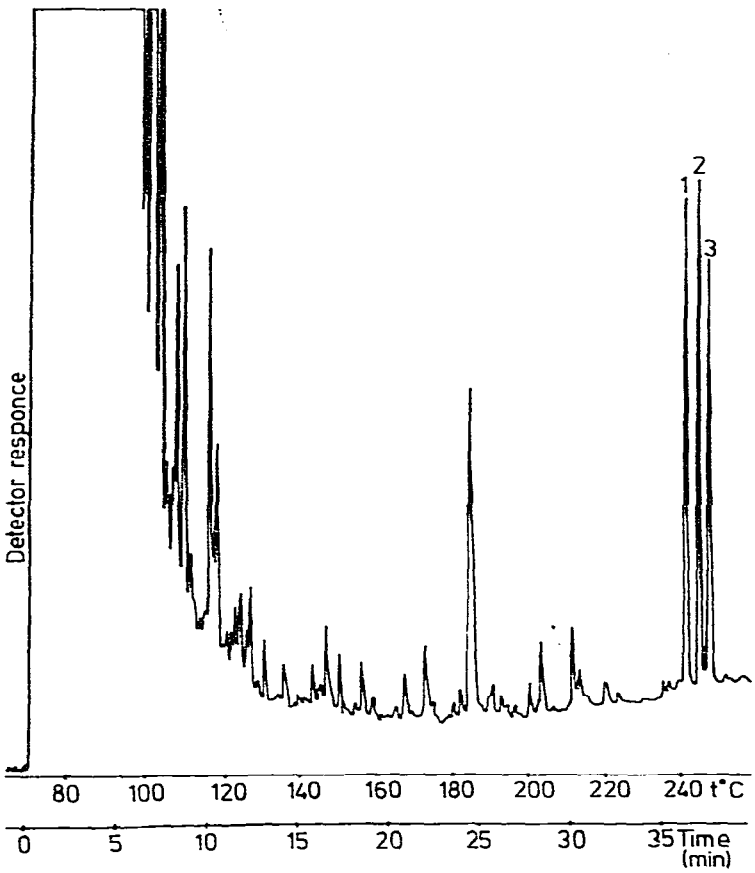

Fig. 3. Chromatogram of a sample containing 10 ng of codeine (peak 1), 10 ng of ethylmorphine (peak 2) and 10 ng of morphine (peak 3) after flash-heater silylation. Column temperature: 70° at start, programmed at 5°/min.

On the basis of these results the derivatization reagent and the sample can be injected directly into a gas chromatograph equipped with a capillary column. Less time is required for forming derivatives, and also there is no chance of the hydrolysis which sometimes occurs with certain derivatives in damp atmospheres. The method can be used successfully for the identification of complex mixtures of narcotic drugs and also for the quantitative analysis of drugs in the nanogram range.

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