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Note

Quantitative gas–liquid chromatography of amphetamine, ephedrine, codeine and morphine after on-column acylation

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The technique of on-column derivatization in gas–liquid chromatography (GLC) has primarily been used to provide additional data for drug identification and has had limited application in quantitative analysis. Most of the work has dealt with on-column silylation, and recently quantitative on-column silylation methods have been reported for cannabinoids[1] and morphine[2]. N,O-Bis(trimethylsilyl)acetamide (BSA) and N-(trimethylsilyl)imidazole (TMSIM), respectively, were used as silylating reagents.

Direct acylation is usually possible for drugs possessing an alcoholic or phenolic hydroxyl group and for drugs that are primary or secondary amines. However, only a few on-column acylation methods have been described, and these have been developed for the purpose of drug identification. Acetyl and propionyl derivatives of various narcotic analgesics have been formed by injecting the drug solution and the acid anhydride into the gas chromatograph[3]. In a mass screening program, amphetamine and methamphetamine have been confirmed as their trifluoroacetamide derivatives using trifluoroacetic anhydride as acylating reagent[4]. The method has recently been modified to include methadone and its major metabolite[5].

When on-column acylation techniques are applied, N-acylimidazoles offer considerable advantages over acyl chlorides and acid anhydrides because no acids are released in the chromatographic system, the relatively inert imidazole being the by-product of the reaction. Fluoroacyl reagents produce stable, volatile derivatives ideal for gas chromatography and with excellent electron capture sensitivity.

The purpose of the present investigation is to show that on-column acylation methods may be successfully used in quantitative analysis. Amphetamine (A), ephedrine (E), codeine (C) and morphine (M) were selected as model substances since these drugs represent a variety of functional groups undergoing acylation reactions. Trifluoroacetyl (TFA) and heptafluorobutyryl (HFB) derivatives of each drug were formed quantitatively and reproducibly by simultaneously injecting the acylating reagent and the drug solution into the gas chromatograph.

EXPERIMENTAL

Materials

The compounds amphetamine, ephedrine, codeine and morphine were of pharmacopoeial grade and were supplied by Norsk Medisinaldepot (Oslo, Norway). Analytical grade benzene and ethyl acetate were obtained from E. Merck (Darmstadt, G.F.R.). N-(Trifluoroacetyl)imidazole (TFAI) and N-(heptafluorobutyryl)imidazole (HFBI) (Pierce, Rockford, Ill., U.S.A.) were used as acylating reagents. The internal standards (I.S.) selected for the GLC determinations, *n*-tetradecane (C_{14}), *n*-hexadecane (C_{16}) and *n*-docosane (C_{22}), were purchased from Koch-Light (Colnbrook, Great Britain).

Stock solutions of each drug and of each internal standard were prepared of concentrations 5 mg/ml and 2.5 mg/ml respectively. Amphetamine, ephedrine, C_{14} and C_{16} were dissolved in benzene, while the solvent for codeine, morphine and C_{22} was ethyl acetate.

Gas chromatography

A Fractovap 2300 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a flame ionization detector (FID) was used. The glass column (2 m × 2 mm I.D.) was packed with 3% SE-30 on Supelcoport (80–100 mesh). The flow-rate of the nitrogen carrier gas was maintained at 30 ml/min. Other operating conditions and the internal standards used are given in Table I.

TABLE I

GLC PERFORMANCE DATA

A-TFA = N,N-bis(trifluoroacetyl)amphetamine, A-HFB = N,N-bis(heptafluorobutyryl)amphetamine, E-TFA = N,O-bis(trifluoroacetyl)ephedrine, E-HFB = N,O-bis(heptafluorobutyryl)ephedrine, C-TFA = O-trifluoroacetylcodeine, C-HFB = O-heptafluorobutyrylcodeine, M-TFA = O,O-bis(trifluoroacetyl)morphine and M-HFB = O,O-bis(heptafluorobutyryl)morphine.

Compound	*I.S.*	*Attenuation*	*Temperature (°C)*		*Derivative*
			Injector	*Column*	
				125	A-TFA
Amphetamine	C_{14}	10 × 128	225		
				130	A-HFB
				150	E-TFA
Ephedrine	C_{16}	10 × 128	225		
				150	E-HFB
				215	C-TFA
Codeine	C_{22}	10 × 64	300		
				220	C-HFB
				210	M-TFA
Morphine	C_{22}	10 × 64	300		
				220	M-HFB

Reproducibility of quantitative analysis after derivatization

Table II shows the composition of the solutions used to study the reproducibility. An amount of 1 μl of a solution was drawn into a syringe (Hamilton 701-N) followed by 2 μl of the acylating reagent, TFAI or HFBI. The mixture was injected

TABLE II

QUANTITATIVE GLC DATA FOR AMPHETAMINE, EPHEDRINE, CODEINE AND MORPHINE AFTER ON-COLUMN ACYLATION

Derivative	*Retention time (min)*	*Reproducibility test*				*Calibration graph*		
		Concentration (mg/ml)		*Peak-height ratio, derivative: I.S.*	*R.S.D. (%)*	*Concentration range (mg/ml)*	*Regression line* $y = ax + b$	*Correlation coefficient*
		Drug	*I.S.*					
A-TFA	3.2	0.75	0.50	1.537	2.4	0.10–1.00	$y = 2.162x - 0.057$	0.9988
A-HFB	3.2	0.75	0.50	1.402	1.5	0.10–1.00	$y = 1.960x - 0.075$	0.9972
E-TFA	2.8	1.00	1.00	1.428	2.3	0.10–1.00	$y = 1.417x - 0.001$	0.9956
E-HFB	3.4	0.75	1.00	0.683	2.2	0.10–1.00	$y = 0.968x - 0.032$	0.9968
C-TFA	8.6	1.00	0.50	0.601	2.8	0.25–2.50	$y = 0.639x - 0.097$	0.9982
C-HFB	8.3	1.00	0.50	0.701	1.1	0.10–2.50	$y = 0.774x - 0.054$	0.9981
M-TFA*	9.4	1.50	0.50	1.377	1.9	0.25–1.50	$y = 0.920x - 0.107$	0.9985
M-HFB	8.6	1.50	0.50	1.165	1.6	0.25–2.50	$y = 0.820x - 0.064$	0.9991

* Only injections giving a single derivative peak were included in the calculations.

into the gas chromatograph and the peak-height ratio of the drug derivative to the internal standard was calculated. The mean and the relative standard deviations (R.S.D.) of ten assays were calculated.

Calibration graphs

Series of four to seven standard solutions were prepared to give a concentration range of each drug of 0.10–2.50 mg/ml. The concentration of the appropriate internal standard was the same as in the reproducibility test. The standard solutions were injected into the gas chromatograph as described above. Five assays of each solution were carried out and the regression line and the correlation coefficient were calculated.

RESULTS AND DISCUSSION

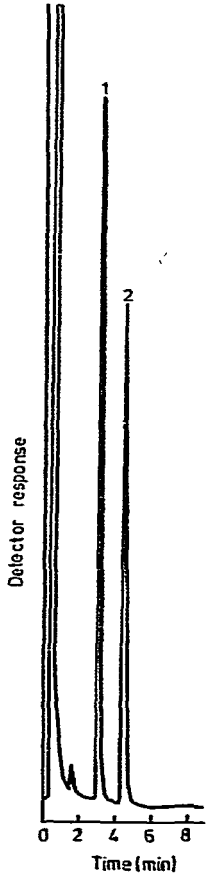
The use of on-column acylation techniques requires little time for forming the derivatives and the reagent cost is greatly reduced. The optimal amount of reagent required to obtain complete reaction was found to be 2 μl under the operating conditions used. No change in the yield or in the retention time of the drug derivative could be observed upon further increase in the volume of the reagent injected. Decomposition and contamination of the reagent may occur, but in our experience the acylating potential is not significantly affected. However, interfering peaks may sometimes cause problems during gas chromatography. Clean chromatograms are obtained by using freshly distilled reagents stored in small, tightly capped dark bottles.

Typical chromatograms obtained after on-column acylation are shown in Figs. 1 and 2. Each drug was converted into a single, well defined derivative possessing a distinct, narrow peak with a reproducible retention time. Furthermore, no parent compound could be detected and the derivatization reaction was therefore considered to be complete. However, when TFAI and morphine were introduced simultaneously into the gas chromatograph, a mixture of mono- and bis(trifluoroacetyl)morphine

was sometimes formed. When incomplete derivatization occurred, an extra peak could be seen on the chromatogram and the peak-height ratio of M-TFA:C_{22} decreased. Consequently, these injections were omitted from the quantitative tests.

The quantitative GLC data are summarized in Table II. The R.S.D. values obtained from the reproducibility tests varied from 1.1 to 2.8%. It appears that for each drug the HFB derivative showed a better reproducibility than the corresponding TFA derivative. The calibration graphs ($y = ax + b$) were calculated according to the method of least squares, relating y, the peak-height ratio of the drug derivative to the internal standard, to x, the concentration of drug solution (mg/ml). The regression lines were linear in the given concentration range, with correlation coefficients varying from 0.9956 to 0.9991.

On the basis of these results it is concluded that the method of on-column acylation is suitable for quantitative determination of the selected substances. Since



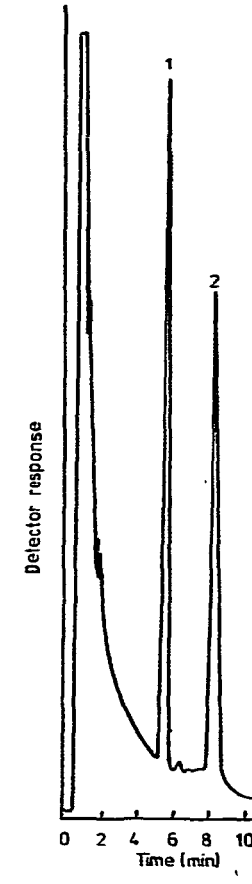


Fig. 1. Gas chromatogram of 0.75 μg of amphetamine after on-column acylation. Peaks: 1 = HFB-amphetamine; 2 = tetradecane (I.S.). Chromatographic conditions as in Table I.

Fig. 2. Gas chromatogram of 1.0 μg of codeine after on-column acylation. Peaks: 1 = docosane (I.S.); 2 = HFB-codeine. Chromatographic conditions as in Table I.

the presented method is simple, rapid and reliable, it deserves a future in quantitative pharmaceutical analysis. Further work on drugs in biological material is directed towards on-column derivative formation in combination with electron capture detection.

REFERENCES

1 K. E. Rasmussen, *J. Chromatogr.*, 114 (1975) 250.
2 K. E. Rasmussen, *J. Chromatogr.*, 120 (1976) 491.
3 S. J. Mulé, *Anal. Chem.*, 36 (1964) 1907.
4 N. C. Jain, T. C. Sneath and R. D. Budd, *Clin. Chem.*, 20 (1974) 1460.
5 N. C. Jain, R. D. Budd, W. J. Leung and T. C. Sneath, *J. Chromatogr. Sci.*, 14 (1976) 293.