Journal of Chromatography, 234 (1982) 107–120
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,188

GLASS CAPILLARY COLUMN GAS CHROMATOGRAPHY OF PHENOLALKYLAMINES AFTER FLASH-HEATER DERIVATIZATION USING A DOUBLE INJECTION TECHNIQUE

ASBJØRG S. CHRISTOPHERSEN, EGIL HOVLAND and KNUT E. RASMUSSEN*
Department of Pharmaceutical Chemistry, Institute of Pharmacy, P.O. Box 1068, Blindern, Oslo 3 (Norway)
(Received June 1st, 1981)

SUMMARY

Glass capillary column gas chromatography has been used for quantitative and qualitative analyses of some phenolalkylamines. The compounds are converted into the corresponding N-trifluoroacetyl-O-trimethylsilyl derivatives using a double injection technique. The phenolalkylamines are first flash-heater trimethylsilylated with N-methyl-N-(trimethylsilyl)trifluoroacetamide injected together with the sample, followed by N-acylation with N-methyl-bis(trifluoroacetamide) in a second injection. The N-acylation occurs on-column. The identity of the derivatives was confirmed by gas chromatography–mass spectrometry. The phenolalkylamines were also analysed using on-column derivatization combined with a packed column and a double injection technique. Several parameters were studied for both methods in order to get optimal response with good reproducibility. The injection technique proved to be the most important parameter in this study and had to be carefully controlled. For the quantitative analyses, the linearity, reproducibility and lower detection limit were controlled. A concentration range of 5–50 μg/ml was investigated using the capillary column and 25–1000 μg/ml for the packed column. The main advantages of this derivatization method are its speed and simplicity, which result in a substantial saving of time.

INTRODUCTION

High-resolution gas chromatography (GC) is finding increasing application for analyses of drugs and related compounds. The wide applicability and acceptance of this method have recently been summarized in a review article[1]. Although efforts to prepare adsorption-free columns have been made, the use of derivatization for compounds containing functional polar groups is still recommended, especially for analyses at the nanogram level.

The flash-heater derivatization technique has earlier been applied to glass capillary columns[2–4]. The reaction occurs in the heated injection port following simultaneous injection of the sample and the reagent. The method is applicable to heat-

0021-9673/82/0000–0000/$02.75

stable compounds that react rapidly with the derivatization reagent to form a single product.

Derivatization of compounds containing different functional groups may give two or more products, as the available reagents do not have optimum properties for reaction with different acceptor groups. Two products were obtained after flash-heater silylation of *p*-aminobenzoic acid[3]. A solution to the problem is to derivatize in two stages with different specific reagents. This has earlier been described for pre-column derivatization of steroids[5], amino acids[6] and catecholamines[7].

N-Methyl-bis(trifluoroacetamide) was developed by Donike for selective acylation of amine functions in the presence of hydroxyl and carboxyl groups[8]. Quantitative derivative formations were obtained if these groups were first trimethylsilylated[9,10].

The purpose of the present investigation was to determine whether compounds containing different functional groups could be quantitatively flash-heater derivatized with two specific reagents. The method was optimized for capillary columns. Some phenolalkylamines were selected as model substances and reagents capable of forming trimethylsilylderivatives were studied, followed by N-acylation with N-methyl-bis(trifluoroacetamide) in a second injection.

A packed column GC method with double on-column derivatization was also developed for the phenolalkylamines. When using a double injection technique, the reactions appear to be quantitative and reproducible for both column methods, giving the corresponding N-trifluoroacetyl-O-trimethylsilyl derivatives. The influence of the injection technique, the injection port temperature, the initial column temperature and the reagent volumes were studied.

For quantitative analyses, the linearity, reproducibility and lower detection limit were evaluated, and the results from both column methods were compared.

MATERIALS AND METHODS

Reagents

Phenylephrine hydrochloride, synephrine, isoproterenol sulphate and ephedrine were supplied by Norsk Medisinaldepot (Oslo, Norway). *p*-Hydroxyephedrine hydrochloride was obtained from Aldrich (Beerse, Belgium) and etilefrin hydrochloride was a gift from Boehringer Ingelheim (Ingelheim, G.F.R.). Nonadecane and eicosane were purchased from Koch-Light (Colnbrook, Great Britain). N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and N-methyl-bis(trifluoroacetamide) (MBTFA) in 5-ml vials were purchased from Fluka AG (Buchs, Switzerland). Ampoules (1 ml) of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), N-trimethylsilylimidazole (TSIM) and Sylon BTZ (mixed BSA, TSIM and trimethylchlorosilane) were purchased from Supelco (Bellefonte, PA, U.S.A.). Analytical grade dimethylformamide (DMF) and pyridine were obtained from E. Merck (Darmstadt, G.F.R.).

Stock standard solutions of the phenolalkylamines and the alkanes used as internal standards contained 1 mg/ml in DMF. The concentration of the stock standard solutions used for packed column analyses was 2 mg/ml in DMF. The concentrations of the compounds available as salts were calculated as bases.

Gas chromatography

A Fractovap 2900 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a flame ionization detector and a capillary column splitless injector was used. The glass capillary column (16 m × 0.35 mm I.D.) (H. and J. Jaeggi, Trogen, Switzerland) was wall-coated with OV-1. The injection port temperature was 275°C, and the samples were injected at an oven temperature of 130°C. The temperature was programmed at 10°/min up to 220°C. Nitrogen was used as 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 sensitivity setting varied from 32 to 8, and a Spectra-Physics Autolab Minigrator was connected to the gas chromatograph for peak-area measurements. The samples were injected with a 5-μl Precision Sampling GC-130 microsyringe equipped with a 7.5-cm needle. The glass liner in the splitless injector was cleaned every other day.

A Fractovap 2300 gas chromatograph (Carlo Erba, Milan, Italy) equipped with a flame ionization detector was used with a packed glass column. The column (1 m × 2 mm I.D.) was packed with 3% SE-30 on Supelcoport (80–100 mesh). The injection port temperature was 275°C, and the samples were injected at an oven temperature of 130°C. The temperature was programmed at 10°/min up to 200°C. The flow-rate of the nitrogen carrier gas was 30 ml/min, and the sensitivity setting varied from 10 × 1 to 10 × 8.

Gas chromatography–mass spectrometry (GC–MS)

GC–MS was carried out using a Micromass 7070F mass spectrometer (VG-Micromass, Altrincham, Great Britain) combined with a Fractovap 4200 gas chromatograph (Carlo Erba, Milan, Italy). The glass column (1 m × 2 mm I.D.) was packed with 3% SE-30 on Supelcoport (80–100 mesh).

Influence of the injection technique

A 1-μl volume of a test solution containing 50 ng of phenylephrine and 50 ng of nonadecane (internal standard) was injected together with 2 μl of MSTFA, followed by 2 μl of MBTFA in a second injection. The peak-area ratios were studied when the time between the two injections varied from 15 to 45 sec. The samples were injected splitless. The splitter was closed before the first injection and re-opened after the second injection (splitless time). This time was varied from 45 to 75 sec, and the peak-area ratios were calculated. The derivatization reaction was also studied when MBTFA was injected with MSTFA and the sample in one injection.

A 1-μl volume test solution containing 500 ng of phenylephrine and eicosane (internal standard) was injected on the packed column with 2 μl of MSTFA; 2 μl of MBTFA was injected in a second injection. The time between the two injections was varied from 15 to 60 sec. The peak-height ratios were calculated.

Testing of derivatization reagents and the influence of their volumes

Four different silylation reagents were tested: TSIM, Sylon BTZ, BSTFA and MSTFA and dilutions of MSTFA in pyridine (1:1) and DMF (1:1). MBTFA was used for the acylation reaction, undiluted and diluted with pyridine (1:1) and DMF (1:1). The concentration of the test solution was 50 μg/ml of phenylephrine and nonadecane.

The peak-area ratios were calculated after injection of 1, 2 and 3 μl of MSTFA

and 1 μl of the test solution, followed by 1, 2 and 3 μl of MBTFA in a second injection.

The different silylation reagents were also tested for on-column derivatization combined with the packed column.

Choice of initial column temperature

The derivatization reaction was studied by injection of 1 μl of test solution and 2 μl of MSTFA, followed by 2 μl of MBTFA in a second injection. The initial column temperature varied from 120 to 150°C. The peak-area ratios were calculated. The packed column initial temperature varied from 110 to 150°C, and the peak-height ratios were calculated.

Calibration graphs and reproducibility tests

Calibration graphs for the concentration range 5–50 μg/ml were constructed for ephedrine, phenylephrine and synephrine using nonadecane as internal standard (30 μg/ml). A 2-μl volume of MSTFA was injected splitless with 1 μl of the sample, followed in a second injection by 2 μl of MBTFA after 20–25 sec. The splitter was re-opened 50 sec after the first injection. The linearity was investigated up to 200 μg/ml.

For the packed column method, a calibration graph for phenylephrine was constructed for the concentration range 25–1000 μg/ml using eicosane as internal standard (500 μg/ml). A period of 20–25 sec was used between the two injections.

Five assays on each solution were carried out and the regression lines and the correlation coefficients were calculated for both methods.

For the reproducibility tests, solutions containing 10 and 50 μg/ml of ephedrine, phenylephrine and synephrine and 30 μg/ml of the internal standard were analysed (capillary column). Solutions containing 50 and 1000 μg/ml of phenylephrine and 500 μg/ml of the internal standard were analysed for the packed column method. The mean and the relative standard deviation (R.S.D.) of ten assays were calculated.

RESULTS AND DISCUSSION

The influence of the injection technique

The injection technique was the most important parameter in this study. Two products were formed when both derivatization reagents were injected together with the sample, but only one peak could be detected using the double injection technique. This was observed with both column methods.

The data in Table I indicate that the reproducibility of the derivatization reactions depends on the time between the two injections, especially for the capillary column method. Incomplete N-acylation and peak tailing may occur with increasing time between the injections. A period of 20–25 sec was therefore used for both columns.

The results were also influenced by the time when the splitter was closed (splitless time). From Table I it can be seen that poor reproducibility is obtained with increasing splitless time. For the quantitative analyses, the splitter was re-opened 50 sec after the first injection.

TABLE I

EFFECT OF THE TIME BETWEEN THE TWO INJECTIONS AND THE SPLITLESS TIME

The electrometer used for Tables I, II, V and VI is different from the one used for Tables III, VII and VIII.

Time between injections (sec)	*Capillary column*		*Packed column*	
	Peak-area ratio ($\bar{x}$, $n = 5$)	*R.S.D.* (%)	*Peak-height ratio* ($\bar{x}$, $n = 5$)	*R.S.D.* (%)
15	1.08	3.9	1.21	2.0
30	1.06	2.8	1.22	2.0
45	1.09	11.8	1.18	2.6
60			1.17	3.0
Splitless time (sec)				
45	1.09	2.4		
60	1.06	2.8		
75	1.03	4.6		

Derivatization studies with different reagents

Different reagents as TSIM, Sylon BTZ, BSTFA and MSTFA were investigated to find the most effective silylation reagent. The reagents were tested in combination with the acylation reagent and also for complete silylation of the phenolalkylamines. For all these reagents, two peaks were obtained when the acylation reagent was omitted. Non-quantitative reaction of amino groups in the complete silylation of catecholamines has earlier been reported for pre-column derivatization. The secondary amine groups are especially difficult to attack and acylation reaction has therefore been preferred[11]. TSIM and Sylon BTZ are the recommended reagents for all hydroxyl groups, but these reagents could not be used for flash-heater derivatization in combination with MBTFA. Broad extra peaks were obtained and, after a few injections, dark particles appeared in the injector part of the capillary column with a drastic loss of column efficiency. The injector part of the packed column also became dark and had to be repacked. MSTFA and BSTFA both seemed to react satisfactorily in combination with MBTFA. Of the two silylation reagents, MSTFA seemed to react more quantitatively than BSTFA and also with a noticably lower R.S.D. (Table

TABLE II

MSTFA COMPARED WITH BSTFA FOR TRIMETHYLSILYLATION

Reagent	*Capillary column*		*Packed column*	
	Peak-area ratio ($\bar{x}$, $n = 5$)	*R.S.D.* (%)	*Peak-height ratio* ($\bar{x}$, $n = 5$)	*R.S.D.* (%)
MSTFA	1.06	2.8	1.20	1.5
BSTFA	0.79	9.2	1.17	3.4

II). The difference was most marked for the capillary column and consequently MSTFA was selected as silylating reagent.

The separation of the N-TFA-O-TMS derivatives of a mixture of the phenolalkylamines is shown in Fig. 1. The trimethylsilylacyl derivatization combined with a glass capillary column is well suited for the identification of these compounds.

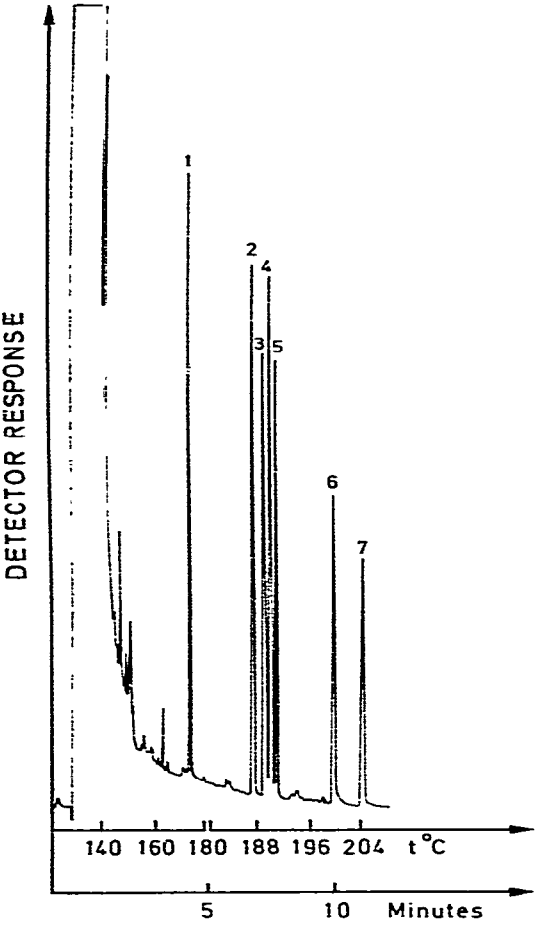


Fig. 1. Chromatogram of a sample containing 50 ng each of ephedrine (1), phenylephrine (2), etilefrin (3), synephrine (4), *p*-hydroxyephedrine (5) and isoproterenol (7) after flash-heater silylation followed by on-column acylation. Peak 6 is 50 ng of the internal standard eicosane. The temperature was programmed at 10°/min from 130 to 180°C then at 4°/min to 220°C. For other chromatographic conditions, see text.

Influence of the reagent volumes

The influence of the amount of the reagent volumes is shown in Table III. It is apparent that at least 2 μl of each reagent must be injected to obtain complete reaction. This volume also gave the best precision. With increasing volumes, the probability of interfering peaks will increase.

TABLE III

EFFECT OF VARIOUS REAGENT VOLUMES INJECTED (CAPILLARY COLUMN)

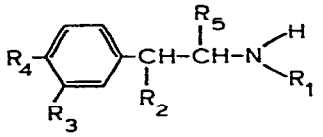
Volume (μl)	*MSTFA varied, 2 μl MBTFA*		*MBTFA varied, 2 μl MSTFA*	
	Peak-area ratio ($\bar{x}$, n = 5)	*R.S.D. (%)*	*Peak-area ratio ($\bar{x}$, n = 5)*	*R.S.D. (%)*
1	0.91	7.6	0.90	4.2
2	0.99	2.4	0.99	2.4
3	0.97	2.5	0.93	4.5

GC–MS investigation of derivatives

The derivatives were identified by GC–MS, and the major ions are listed in Table IV. These data support the formation of N-trifluoro-O-trimethylsilyl derivatives of the phenolalkylamines, and the ions are consistent with previously published data[9]. Only one peak could be detected when using the double injection technique, and it was concluded that the reaction was complete. Two products were obtained from phenylephrine when the second injection with MBTFA was omitted, and both

TABLE IV

PRINCIPAL MASS SPECTRAL DATA OF PHENOLALKYLAMINE DERIVATIVES



Compound	R_1	R_2	R_3	R_4	R_5	*Major ions with relative intensity in parentheses*			
									M − 15
Ephedrine	CH_3	OH	H	H	CH_3	73 (100)	179 (83)		318 (0.5)
Etilefrin	C_2H_5	OH	OH	H	H	73 (98)	267 (100)		406 (1.8)
Synephrine	CH_3	OH	H	OH	H	73 (84)	267 (100)		392 (0.9)
p-Hydroxyephedrine	CH_3	OH	H	OH	CH_3	73 (67)	267 (100)		406 (0.7)
Isoproterenol	C_3H_7	OH	OH	OH	H	73 (82)	267 (1.6)	355 (100)	508 (0.9)
Phenylephrine	CH_3	OH	OH	H	H	73 (99)	267 (100)		392 (1.7)
Phenylephrine (only with MSTFA)									
Main derivative						73 (37)	116 (100)	267 (2.1)	368 (1.4)
By-product						44 (100)	73 (96)	267 (28)	296 (2.6)
Phenylephrine (MSTFA and MBTFA in one injection)									
Main derivative						73 (100)	267 (82)		392 (1.5)
By-product)						73 (100)	291 (25)		416 (1.5)

were identified (Table IV). The mass spectral analysis of the main product indicated the presence of a third trimethylsilyl group introduced on the secondary amine nitrogen. A by-product with shorter t_r was identified as O,O-bis(trimethylsilyl)phenylephrine. Two products were obtained when MBTFA and MSTFA were simultaneously injected with phenylephrine. The main peak was identified to be the same product as the one obtained after a double injection technique, while the data obtained from the by-product indicated the formation of N,O-bis(trifluoroacetyl)-O-trimethylsilyl phenylephrine.

Column temperature effect

The effect of different initial column temperatures is shown in Table V. No significant difference in the peak-areas or the peak-height ratios was observed, but the R.S.D. was increased for the highest temperature. At 150°C, reduced response and broader peaks were observed for the capillary column. An initial column temperature of 130°C was used for both methods, which gave a satisfactory R.S.D. In order to obtain good solvent effect and to minimize the capillary column analysis time, an initial column temperature of 15–30°C below the boiling point of the solvent has been recommended[12]. The boiling point of DMF is 153°C. The results were not influenced by an isothermal period of 3 min before the start of the capillary column temperature programming.

The derivatization reaction was independent of the injection port temperature in the range 250–300°C.

TABLE V

THE EFFECT OF INITIAL COLUMN TEMPERATURE

Capillary column			*Packed column*	
Initial column temperature (°C)	*Peak-area ratio ($\bar{x}$, $n = 5$)*	*R.S.D. (%)*	*Peak-height ratio ($\bar{x}$, $n = 5$)*	*R.S.D. (%)*
110			1.15	1.9
120	1.05	3.4		
130	1.06	2.8	1.17	2.0
140	1.04	3.9		
150	1.10	6.9	1.16	6.0

Dilution of the derivatization reagents

With direct injection of derivatization reagent, by-products or generated impurities can interfere with the peak of interest at lower concentration levels[4]. Direct injection of the reaction medium after pre-column derivatization with MSTFA and MBTFA has been recommended[9]. In this investigation, excess reagents and by-products did not affect the packed column analyses significantly. Fig. 2 shows that 25 ng of phenylephrine could easily be detected without interferences. With the capillary column analyses, excess reagent by-products caused interference with sample sizes below 1 ng. Fig. 3 shows an injection of 1 ng each of ephedrine, phenylephrine and synephrine and 5 ng of nonadecane. Experiments with reagent dilutions did not make analyses at lower concentration levels possible as decreased peak-area ratios were

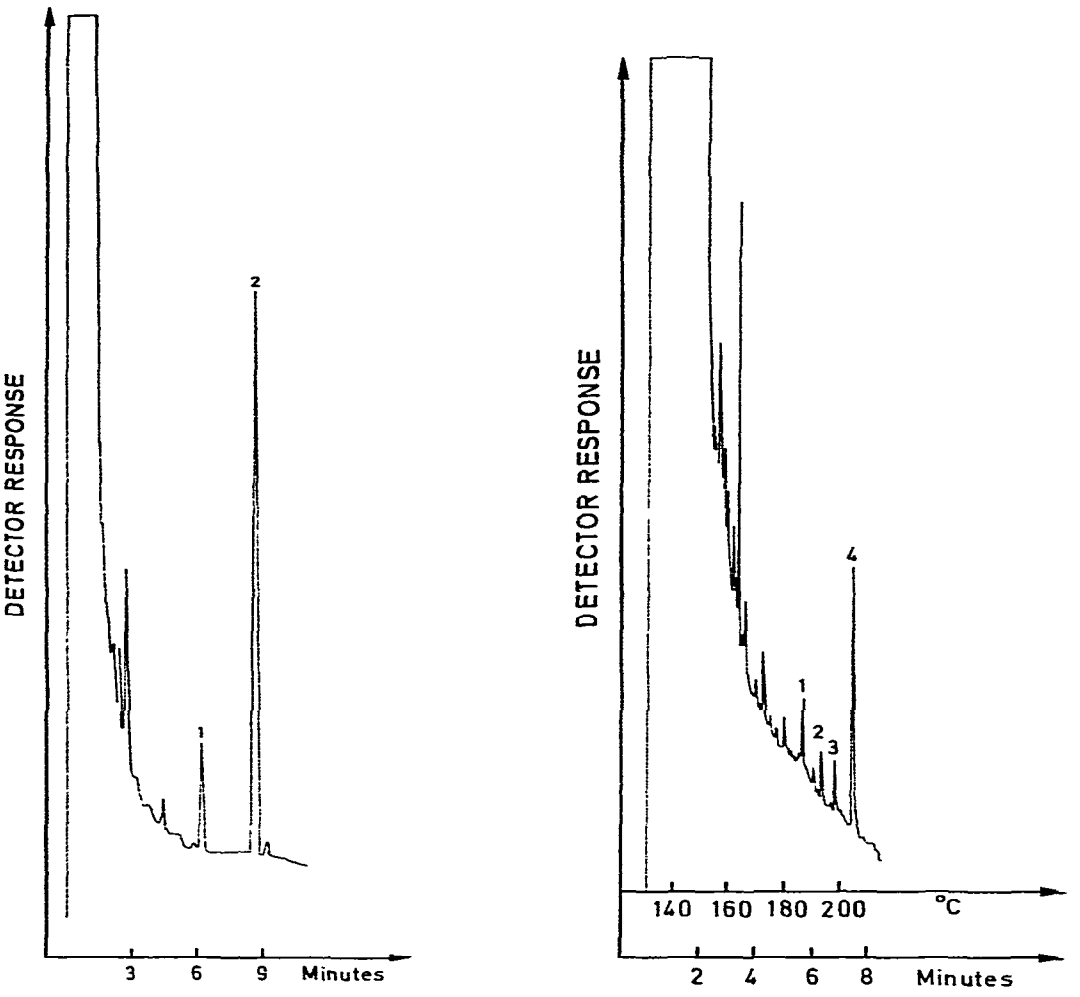


Fig. 2. Packed column chromatogram of a sample containing 25 ng of phenylephrine (1) and 125 ng of eicosane (2) as internal standard, after on-column silylation and acylation. For chromatographic conditions, see text.

Fig. 3. Chromatogram of a sample containing 1 ng each of ephedrine (1), phenylephrine (2) and synephrine (3) after flash-heater silylation followed by on-column acylation. Peak 4 is 5 ng of the internal standard nonadecane. For chromatographic conditions, see text.

observed (Table VI). As shown in Fig. 4, the reagent dilutions also gave unsatisfactory results when used for packed column derivatization. The same sample solution was injected with undiluted MSTFA and MBTFA, and with both reagents diluted with DMF (1:1).

The influence of the syringe needle handling technique

Previous papers have reported that the results obtained from injections into vaporizing GC injectors are dependent on the needle handling technique[13,14]. The technique used in our investigations is as follows:

TABLE VI

THE EFFECT OF REAGENT DILUTIONS (CAPILLARY COLUMN)

Reagent dilution	*Peak-area ratio*	*Comments*
MSTFA MBTFA	1.06	
MSTFA–DMF (1:1) MBTFA	1.03	double peaks occasionally observed
MSTFA MBTFA–DMF (1:1)	0.91	
MSTFA–DMF (1:1) MBTFA–DMF (1:1)	0.90	
MSTFA–pyridine (1:1) MBTFA	0.85	

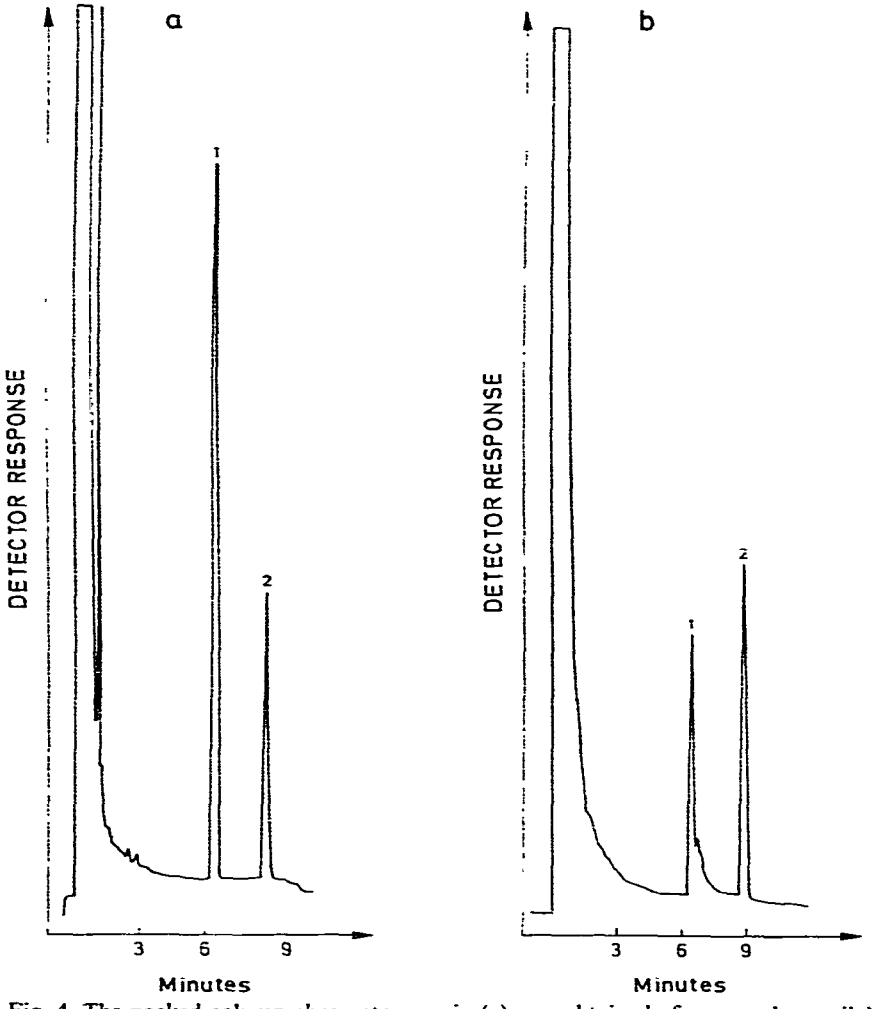


Fig. 4. The packed column chromatogram in (a) was obtained after on-column silylation with MSTFA followed by on-column acylation with MBTFA. The sample contained 1 μg of phenylephrine (1) and 0.5 μg of eicosane (2) as internal standard. Chromatogram (b) was obtained when the same solution was injected with a DMF dilution of both reagents (1:1). For chromatographic conditions, see text.

(1) The needle is filled with 1 μl of reagent, then 1 μl of sample and 1 μl of reagent. The solutions are withdrawn into the syringe barrel and injected without preheating over a period of 5 sec ("cold needle" technique). We have found previously that the rate of sample injection is important in order to ensure a uniform rate of sample vaporization[3]. Previously described techniques for handling syringe needles[13,14] were studied in this investigation.

(2) Same as (1), but injection after 5 sec of needle preheating ("hot needle" technique).

(3) Same as (1), but injection without withdrawing the sample from the needle ("filled needle" technique).

(4) Filling the needle with all reagent volume (2 μl) before picking up the sample.

(5) Filling the needle with sample before picking up the reagent (2 μl).

The data in Table VII show that the highest peak-area ratio combined with lowest R.S.D. was obtained for the usual needle handling technique ("cold needle"). In Grob's investigation, the "hot needle" technique was found to be superior to other methods, but most of his experiments were performed on alkanes[13,14]. Reduced syringe needle discrimination was observed for larger sample volumes[14]. Large sample volumes were also injected in this study. The results were most influenced by the syringe needle length. A microsyringe equipped with a 7.5-cm needle had to be used for Fractovap 2900 GC injections. With an ordinary 5-cm needle, double peaks were obtained.

TABLE VII

EFFECT OF DIFFERENT SYRINGE NEEDLE HANDLING TECHNIQUES

Technique	*Peak-area ratio* ($\bar{x}$, $n = 5$)	*R.S.D.* (%)
Cold needle (1)	0.97	2.4
Hot needle (2)	0.92	2.3
Filled needle (3)	0.93	3.8
All reagent behind the sample (4)	0.99	4.9
All reagent in front of the sample (5)	0.90	4.0

Calibration graphs

Calibration graphs in the concentration range 5–50 μg/ml were constructed for ephedrine, phenylephrine and synephrine in order to check the linearity of the derivatization method (Table VIII). The linearity was also checked up to 200 μg/ml and no change was observed. Fig. 5 shows a chromatogram of a sample containing 10 ng of the derivatized compounds and 30 ng of nonadecane.

A linear calibration graph was also obtained for phenylephrine in the concentration range 25–1000 μg/ml when analysed on the packed column (Table VIII).

The data from the reproducibility tests also show acceptable results for both columns (Table VIII).

TABLE VIII

DATA FROM THE CALIBRATION GRAPHS AND REPRODUCIBILITY TESTS AFTER DERIVATIZATION

Compounds	*Calibration graph equation over range 5–50 μg/ml and 25–1000 μg/ml*	*Correlation coefficient*	*R.S.D. (%)*		
			10 μg/ml	*50 μg/ml*	*1 mg/ml*
Capillary column					
Ephedrine	$y = 0.029x - 0.074$	0.998	4.2	2.7	
Phenylephrine	$y = 0.036x - 0.116$	0.999	3.9	2.2	
Synephrine	$y = 0.035x - 0.084$	0.997	3.8	2.2	
Packed column					
Phenylephrine	$y = 0.385x + 0.003$	0.999		1.4	1.2

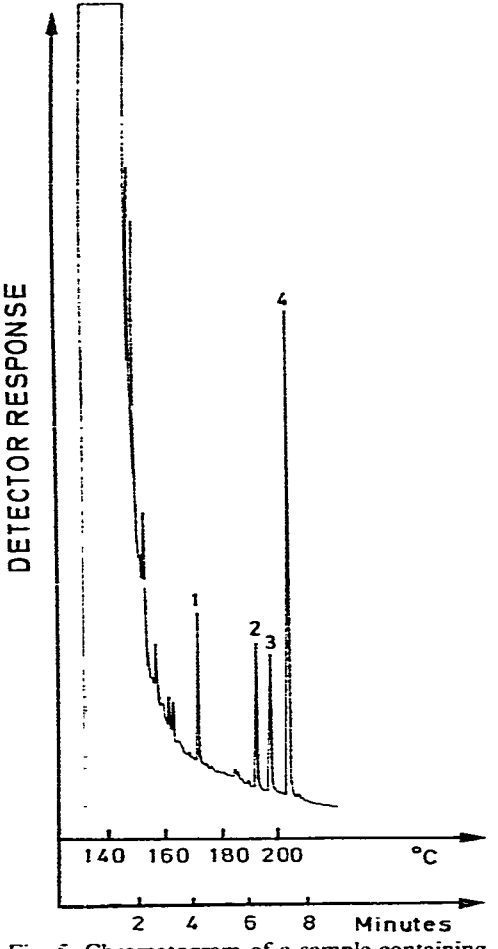
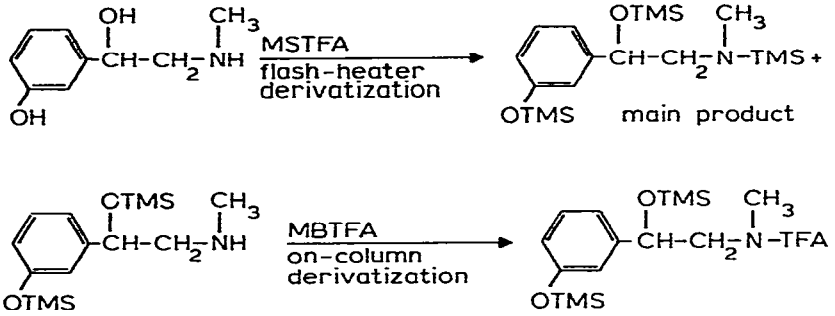


Fig. 5. Chromatogram of a sample containing 10 ng each of ephedrine (1), phenylephrine (2) and synephrine (3) after flash-heater silylation followed by on-column acylation. Peak 4 is 30 ng of the internal standard nonadecane. For chromatographic conditions, see text.

CONCLUSIONS

This study shows that compounds containing both hydroxyl and amino groups can be quantitatively derivatized using specific reagents combined with a double injection technique. The derivatization technique is applicable to both glass capillary and packed columns. With capillary columns, the silylation occurs in the flash-heater of the gas chromatograph. With phenylephrine as model substance the N,O,O-tris-(TMS) derivative was formed together with some of the N,O-bis-(TMS) derivative. The formation of these products was verified by GC–MS. After the injection of MBTFA, all N-TMS groups are replaced by N-TFA and unreacted secondary amine groups are acylated. These reactions occur on-column. The reaction scheme earlier proposed by Donike for pre-column derivatization[9] may also be applied to this study.



With packed column analyses, the silylation and acylation reactions both occur on-column. The main advantages of this derivatization technique are its speed and simplicity, which result in a substantial saving of time. Expensive reagents are saved, when only few microlitres are required for each analysis. The mixture shown in Fig. 1 could not be separated on the packed column. However, for the simplest type of separations, packed columns may be employed if adequate sensitivity is obtained. This column type is simpler to operate than capillary columns and is not so sensitive to the different parameters controlled in this study. The derivatization technique combined with capillary columns is a valuable tool for the identification of complex mixtures and for quantitative analyses of drugs at low concentrations.

ACKNOWLEDGEMENT

Financial support by Norsk Medisinaldepot (Oslo, Norway) is gratefully acknowledged.

REFERENCES

1 W. J. A. VandenHeuvel and J. S. Zweig, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 381.
2 A. S. Christophersen and K. E. Rasmussen, *J. Chromatogr.*, 174 (1979) 454.
3 A. S. Christophersen, K. E. Rasmussen and F. Tønnesen, *J. Chromatogr.*, 179 (1979) 87.
4 A. S. Christophersen and K. E. Rasmussen, *J. Chromatogr.*, 192 (1980) 363.
5 J. A. Luyten and G. A. F. M. Rutten, *J. Chromatogr.*, 91 (1974) 393.

6 J. P. Hardy and S. L. Kerrin, *Anal. Chem.*, 44 (1972) 1497.
7 J.-C. L'Huguenot and B. F. Maume, *J. Chromatogr. Sci.*, 12 (1974) 411.
8 M. Donike, *J. Chromatogr.*, 78 (1973) 273.
9 M. Donike, *J. Chromatogr.*, 103 (1975) 91.
10 G. Schwedt and H. H. Bussemas, *J. Chromatogr.*, 106 (1975) 440.
11 J. D. Nicholson, *Analyst (London)*, 103 (1978) 193.
12 F. J. Yang, A. C. Brown III and S. P. Cram, *J. Chromatogr.*, 158 (1978) 91.
13 K. Grob, Jr. and H. P. Neukom, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 15.
14 K. Grob, Jr. and S. Rennhard, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 627.