CHROM. 12,531

GAS CHROMATOGRAPHY OF EPINEPHRINE AND NOREPINEPHRINE AFTER DERIVATIZATION WITH CHLOROFORMATES IN AQUEOUS MEDIA

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(Received November 12th, 1979)

SUMMARY

Optimal conditions for the reaction of alkyl chloroformates with epinephrine and norepinephrine in aqueous solution have been evaluated. The maximal yield with 0.06 M methyl chloroformate was in the pH range 7.0–8.5. The derivatives formed are isolated by extraction with methylene chloride and gas chromatographed on 3% QF-1 with a nitrogen-selective detector after trimethylsilylation of the alcohol group.

INTRODUCTION

The gas chromatographic analysis of catecholamines necessitates the blocking of the polar groups, e.g., by silylation[1–3], acylation[4,5], perfluoroacylation[6] or a combination of perfluoroacylation and silylation[7–9].

Isolation of catecholamines by conventional batch extraction from biological samples is difficult owing to their polar and amphoteric character and their sensitivity towards oxidation. Adsorption on alumina[10] is used in many methods as a means of purification of the sample. The proteins in plasma are normally precipitated before this step[9,11,12].

Most derivatives are to some extent sensitive to moisture, mainly due to the derivatized phenol groups. The more lipophilic perfluoroacyl derivatives show better stability[13]. However, perfluoroacyl and acyl groups on beta-hydroxyls are also labile[5,14]. By blocking the alcohol group with an alkyl group[11,15,16], this problem may be circumvented.

To reduce the polar character of catecholamines and the risk of oxidation of the catechol structure, and to facilitate their isolation by extraction, acylation in aqueous media has been performed. Originally used in pharmaceutical analyses[17,18], acetic anhydride has been employed in gas[4,5,19] and liquid[20] chromatographic analysis. A two-phase system, using acyl anhydrides in ethyl acetate, has also been shown to be efficient[21]. This method was used in the blocking of phenol groups of catecholamine metabolites prior to derivatization of polar groups in the side-chain[22–26].

Chloroformate reagents react virtually instantaneously with amines in aqueous

media[27]. They have been used in the gas chromatographic analysis of amines[28] and phenols[29] after derivatization in aqueous alkaline solution.

The aim of this study was to develop conditions for the derivatization of catecholamines in aqueous media at an early step stage in the overall procedure and their subsequent analysis by gas chromatography.

EXPERIMENTAL

Instruments

Gas chromatography. A Varian 3700 chromatograph with flame-ionization and thermionic [nitrogen–phosphorus (NP)[30]] detectors was used, equipped with glass columns (150 or 180 × 0.2 cm I.D.). The injector and the detector were maintained at 250° and 300°, respectively, and the column oven at 215–240°. Gas flow-rates for the NP detector were as follows: nitrogen 30, hydrogen 5 and air 175 ml/min. The column packings used are described under *Reagents and chemicals.*

Mass spectrometry. A Varian-MAT 112 mass spectrometer coupled to a Varian 1400 gas chromatograph was used. The glass column (100 × 0.2 cm I.D.) was filled with 3% QF-1 on Chromosorb W HP (80–100 mesh). The helium flow-rate was 20 ml/min. The slit valve to the ion source was opened manually to a pressure of $2 \cdot 10^{-5}$ torr after venting the solvent. The temperatures were injector 230°, column 200–240°, transfer line 250° and ion source 230°. The electron energy was 70 eV and the emission current 1.5 mA.

Reagents and chemicals

Methyl chloroformate was obtained from Aldrich (Milwaukee, Wisc., U.S.A.) and ethyl and isobutyl chloroformate from EGA Chemie (Steinheim am Albuch, G.F.R.).

Acetic and propionic anhydride and hexamethyldisilazane were purchased from Fluka (Buchs, Switzerland) and trimethylchlorosilane from Macherey, Nagel & Co. (Düren, G.F.R.).

Epinephrine hydrogen bitartrate from Fluka and norepinephrine hydrogen chloride from Sigma (St. Louis, Mo., U.S.A.) were used. N-*n*-Butyl and N-*n*-hexyl-norepinephrine, tris and tetracetylepinephrine were synthesized at AB Hässle (Department of Organic Chemistry).

The trichloroethyl carbamate of dibenzylamine was prepared by reaction of equimolar amounts of dibenzylamine (Fluka) and trichloroethyl chloroformate (Aldrich) in methylene chloride in the presence of sodium carbonate. The solution was washed with dilute sulphuric acid and sodium hydroxide solution.

Sodium phosphates and carbonates were used for the preparation of aqueous buffers ($\mu = 1$).

Solvents (analytical-reagent grade) were obtained from Merck, Darmstadt, G.F.R. (methylene chloride), Fisher Scientific, Pittsburgh, Pa., U.S.A. (hexanes) and May & Baker, Dagenham, Great Britain (ethyl acetate).

Column packings. QF-1, 3% on Gas-Chrom Q (100–120 mesh), was obtained from Applied Science Labs. (State College, Pa., U.S.A.) and 3% OV-210 on the same support from Ohio Valley Speciality Chemical (Marietta, Ohio, U.S.A.).

QF-1 (F & M Scientific, Avondale, Pa., U.S.A.), 3% on Chromosorb W HP

(80–100 mesh) (Johns-Manville, Lompoc, Calif., U.S.A.), and 3% OV-210 (Applied Science) on Super Pak 20 M (Analabs, North Haven, Conn., U.S.A.) were prepared by gentle evaporation of a suspension of the support in a solution of the stationary phase in a glass bowl.

The packed columns were conditioned for at least 24 h with a flow of carrier gas at their maximum recommended temperature. Before use they were treated with two 10-μl portions of Silyl-8 (Pierce, Rockford, Ill., U.S.A.).

Determination of catecholamines with methyl chloroformate

A solution of the catecholamines in 0.01 M hydrochloric acid (1.0 ml), plasma (1.0 ml) and 2.0 ml of phosphate buffer (pH 7.4) were mixed with 20 μl of methyl chloroformate for 30 sec. After 5 min the mixture was extracted with 10.0 ml of methylene chloride (containing the trichloroethyl carbamate of dibenyzlamine as internal standard) for 5 min. After centrifugation an aliquot of the organic phase (8.0 ml) was evaporated to dryness under a stream of air at 30°. The residue was treated with silylating reagent (100 μl of hexamethyldisilazane and 20 μl of tri-methylchlorosilane) for at least 90 min at room temperature. The reaction mixture was taken to dryness and reconstituted in ethyl acetate (minimum volume 25 μl). A 1–2-μl volume was taken for analysis by gas chromatography with the NP detector.

Determination of catecholamines after acetylation

An aqueous solution of the catecholamines (4.0 ml) was mixed with 440 mg of sodium hydrogen carbonate or 740 mg of disodium hydrogen phosphate. Acetic anhydride (225 μl) was added in four portions over a period of 2 min. After a total reaction time of 5 min the derivatives were extracted into methylene chloride (10.0 ml). An aliquot (8.0 ml) was evaporated to dryness and silylated with 50 μl of methyl-silyltrifluoroacetamide in the presence of 200 μl of freshly glass-distilled pyridine. After a few minutes the solution was evaporated to dryness with a stream of air and reconstituted in methylene chloride (200 μl, flame-ionization detection) or in ethyl acetate (NP detection).

Determination of catecholamines after propionylation

The method using methyl chloroformate was followed, with the addition of a pH shift step after the propionylation reaction. By adding solid potassium hydroxide the pH was adjusted to 11–12.
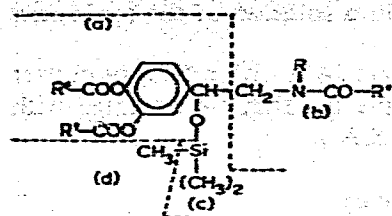
RESULTS AND DISCUSSION

Identification of derivatives formed

The derivatives formed were identified by mass spectrometry after a gas chromatographic separation. The major ions are listed in Table I. Most important from the structural point of view are the ions formed from α-cleavage of the molecular ion (a and b, Table I). The heavy mass ion (a) would be suitable for quantitation by mass fragmentography.

The α-ions of the methyl chloroformate derivatives gave higher relative intensities than those from other alkyl chloroformates. The derivatization conditions involving the use of methyl chloroformate were therefore studied in more detail.

TABLE I

ESSENTIAL MASS SPECTRAL DATA OF CATECHOLAMINE DERIVATIVES

R'	R	Relative intensity of fragments (%) *			
		a	b	c	d (73)
CH$_3$O	H	23 (327)	1 (88)	1	100
CH$_3$O	CH$_3$	55 (327)	37 (102)	2	100
CH$_3$O	n-C$_6$H$_{13}$	69 (327)	13 (172)	3	49
C$_2$H$_5$O	H	3 (355)	1 (102)	—	100
C$_2$H$_5$O	CH$_3$	25 (355)	49 (116)	—	100
C$_2$H$_5$O	n-C$_6$H$_{13}$	21 (355)	27 (186)	—	88
i-C$_4$H$_7$O	H	2 (411)	—	—	100
i-C$_4$H$_7$O	CH$_3$	3 (411)	14 (144)	—	100
i-C$_4$H$_7$O	n-C$_6$H$_{13}$	5 (411)	14 (214)	—	51
CH$_3$	H	10 (295)	4 (72)	1	100
CH$_3$	CH$_3$	9 (295)	5 (86)	1	100
CH$_3$	n-C$_6$H$_{13}$	20 (295)	5 (156)	1	77

* m/e values given in parentheses.

Optimal pH of the aqueous phase

The yield of methyl chloroformate derivatives and the dependence on the pH of the aqueous phase are illustrated in Fig. 1. Maximal yields were obtained in the pH range 7.0–8.5; the yield decreased rapidly at lower pH but more slowly at
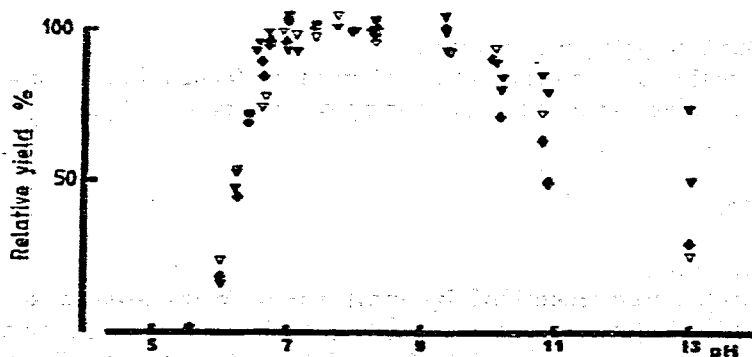
Fig. 1. Yield of methyl chloroformate derivatives versus pH of the aqueous phase. Method: 2.0 ml of catecholamine solution and 2.0 ml of buffer ($\mu = 1$) were used. The pH was measured just before addition of 20 μl of methyl chloroformate. The derivatives were extracted with 5.0 ml of methylene chloride containing 3 μg of the internal standard. A volume of 3.0 ml was taken for silylation. Final volume in ethyl acetate: 300 μl. ♦, Epinephrine (5 μg taken); ▼, norepinephrine (16 μg); ▽, N-n-hexylnorepinephrine (30 μg).

higher pH. The low but more constant yield at lower pH was probably due to blocking of the amino group by a proton, whereas at higher pH the fluctuations were caused by oxidation of the catechol group.

Attempts were also made to block the phenol groups selectively by starting the reaction at a lower pH than that required for optimal yield. The reaction was started with methyl chloroformate, quenched and continued with isobutyl chloroformate after adjusting the pH to about 8. Four derivatives were always obtained in the chromatograms and mass spectral analysis showed the presence of three, two, one and no methyl chloroformate residues and no, one, two and three isobutyl chloroformate residues. If present, the methyl chloroformate residue was alway on the nitrogen, which indicates that the reactivity with amino groups is higher than that with phenol groups[27].

Amount of methyl chloroformate

The relative yields of the derivatives with decreasing amounts of methyl chloroformate are given in Table II. Although 15% of the aqueous phase was plasma, a constant yield was obtained down to 20 μl of the reagent. In the presence of plasma and large amounts of chloroformate (*e.g.*, 100 μl/4.0 ml of aqueous solution) the reaction mixture tended to form a gel, which made the subsequent methylene chloride extraction difficult. The addition of a further 3.0 ml of water overcame this problem. The problem did not arise when the amount of reagent was reduced and the need for additional water could thus be eliminated. The solubility of methyl

TABLE II

EFFECT OF AMOUNT OF METHYL CHLOROFORMATE

Conditions: 1.0 ml of a solution containing 6.3 μg of epinephrine, 20.1 μg of norepinephrine and 35.2 μg of N-*n*-hexylnorepinephrine, was used, with volumes of water and buffer as indicated. The reaction mixture was extracted with 5.0 ml of methylene chloride containing 3 μg of the internal standard. A volume of 3.0 ml of the organic phase was taken for silylation. The final volume in ethyl acetate was 0.25 ml.

Methyl chloroformate (μl)	Plasma (ml)	Water (ml)	Buffer (ml)	Relative yields (%)		
				Epinephrine	Norepinephrine	Hexylnorepinephrine
100	1.0	3.0	2.0	101	100	68
				99	101	92
50	1.0	3.0	2.0	100	99	99
				101	103	96
20	1.0	3.0	2.0	98	95	99
				101	101	114
10	1.0	3.0	2.0	82	79	90
				79	77	85
20	1.0	3.0	2.0	102	100	97
				98	98	69
20	1.0	0	2.0	100	99	102
				98	100	81
20	1.0	0	1.0	99	100	94
				104	105	109
20*	1.0	0	1.0	98	98	97

* Reaction time 10 min.

chloroformate is about 50 $\mu l/4.0$ ml (0.16 M) of phosphate buffer (pH 7.4). Hydrolysis of the chloroformate reagents[27-31] lowered the pH of the reaction mixture. With 0.06 M methyl chloroformate and an initial pH of 7.4, the shift after 5 min was 0.4 pH unit, and 0.7 pH unit when plasma was used. An ionic strength of 1.0 for the buffers was used to counteract the pH drift.

Deproteinization of plasma samples with perchloric acid

Often in the analysis of catecholamines the plasma proteins are removed before the isolation starts. Perchloric acid was used in this study and the effect of this treatment is shown in Table III. It is apparent that considerable losses (30%) of the catecholamines occur in the precipitation reaction compared with the procedure when the reaction is performed directly with the whole plasma sample present. These losses can be reduced to some extent if the precipitate is washed with water (Table III). The main advantages of using the precipitation procedure were a reduction in the number of interfereing peaks in the chromatograms and that the pH shift in the derivatization step was comparable to that seen when water samples were analysed.

TABLE III

YIELDS OF CATECHOLAMINES AFTER TREATMENT OF THE PLASMA SAMPLE WITH PERCHLORIC ACID

Conditions: (1) 0.5, 1.6 and 3.7 μg of the catecholamines; 10.0 ml of methylene chloride with 0.5 μg of the internal standard. Final volume before injection, 100 μl. (2) 1.0 ml of plasma with the amounts of catecholamines given in 1. (3) 1.0 ml of plasma as in 2 was mixed with 0.4 ml of 14% perchloric acid and shaken vigorously for a few seconds before centrifugation for 90 sec at 1000 g. The supernatant was collected and adjusted to pH 7.4 with 0.5 M disodium phosphate. (4) The precipitate from 3 was shaken with 1.0 ml of water, which was collected after centrifugation and treated as above.

No.	Sample	Relative yield (%)		
		Epinephrine	Norepinephrine	N-n-Hexylnorepinephrine
1	Aqueous	100	100	100
2	Plasma	104	84	46
		99	87	50
3	Plasma and perchloric acid	63	61	35
		59	64	37
4	Aqueous wash of the precipitate in 3	18	16	20
		16	15	19

Isolation of chloroformate derivatives from the reaction mixture

The distribution of the chloroformate derivatives of epinephrine between hexane or methylene chloride and phosphate buffer (pH 7.4) was studied. Methylene chloride gave a quantitative recovery (> 95%) of the methyl chloroformate derivative using equal phase volumes. This was also the case with the norepinephrine derivative, showing the change towards a more lipophilic character of the compounds. About 50% of the ethyl derivative and only trace amounts of the methyl chloroformate derivative are extracted into hexane. The isobutyl derivative is recovered quantitatively in the hexane phase.

The yield of the derivatives from the reaction mixture in the presence of plasma

was not affected by the extraction time over a period of 2–60 min shaking. The longer shaking times (30–60 min) resulted in extraneous peaks in the chromatograms. Too vigorous shaking should also be avoided as it caused an emulsion at the interface; this was difficult to break by centrifugation but it could be reduced by stirring with a glass rod. Normally the tubes were inverted manually for a period of 5 min before centrifugation.

Acylation of catecholamines in aqueous media

Acetylation of epinephrine and norepinephrine in aqueous solutions was initially performed according to previously published methods[17,19,22]. The use of large amounts of sodium hydrogen carbonate and acetic anhydride with plasma or urine samples resulted in foaming due to the carbon dioxide evolved. We found that disodium hydrogen phosphate could be substituted for sodium hydrogen carbonate. Inconsistent quantitative results were still obtained, mainly owing to incomplete distribution of the trisacetyl derivative of norepinephrine. In the methylene chloride–phosphate buffer (pH 7.4) system a distribution ratio (D) of 2.5 was found ($D = C_{org}/C_{aq}$).

Propionylation of norepinephrine in aqueous media gives a tris derivative with $D = 15$, in the system discussed above, which means that more than 95% will be extracted into methylene chloride if a phase ratio of 2.5 is used. The propionylation reaction was carried out as described for methyl chloroformate. A 50-μl (0.4-mmole) volume of propionic anhydride was deemed necessary (Table IV) and

TABLE IV

EFFECT OF AMOUNT OF PROPIONIC ANHYDRIDE USED

Method: 2.0 ml of water, 2.0 ml of buffer ($\mu = 1$) and 6.4 μg of epinephrine and 17.6 μg of norepinephrine were mixed with x μl of propionic anhydride. After 5 min the reaction mixture was extracted with 10.0 ml of methylene chloride containing 3 μg of the internal standard; 6.0 ml of the organic phase were evaporated and silylated before redissolving in 100 μl of ethyl acetate.

Volume of propionic anhydride, x (μl)	pH	Peak-height ratio to internal standard	
		Epinephrine	Norepinephrine
10	7.4	0.68	0.73
25	7.4	0.88	0.97
50	7.4	1.0	0.99
10	7.9	0.72	0.73
50	7.9	0.88	0.86

a pH of 7.4 was adequate. The results after inclusion of plasma are shown in Table V. To avoid co-extraction of propionic acid, and its consumption of the silylating reagent, the pH of the reaction mixture was adjusted to 11–12 before extraction with methylene chloride. From Table V it is clear that the recovery of the catecholamines from plasma is quantitative relative to that from water.

Trimethylsilylation prior to gas chromatography

Gas chromatographic analysis of the chloroformate and acyl derivatives without protection of the alcohol group was not successful. The trisacetyl derivative of

TABLE V

RECOVERY OF PROPIONYLATED CATECHOLAMINES FROM PLASMA RELATIVE TO THAT FROM WATER

Amounts of catecholamines used: epinephrine 6.4 μg and norepinephrine 17.6 μg. Solid potassium hydroxide was added to the reaction mixture before extraction with 10.0 ml of methylene chloride containing 3 μg of the internal standard. Final volume in ethyl acetate: 100 μl.

Sample	Amount of propionic anhydride (μl)	Recovery (%)	
		Epinephrine	Norepinephrine
Water	50	103	104
		97	96
Plasma	50	102	102
		100	107
	100	85	91
		100	114

epinephrine was synthesized in its pure form but gave multiple peaks in the chromatograms. The identification of one of these peaks as the dehydrated derivative was confirmed by mass spectral analysis and synthesis. Similar problems were also found with the pure tetraacetyl derivative.

Trimethylsilylation of the trisacyl[19] and the trischloroformate derivatives improved the chromatographic properties considerably. However, when potent silylating agents[32] such as bis(trimethylsilyl)acetamide or methyltrimethylsilyltrifluoroacetamide were used, two peaks were obtained from the chloroformate derivatives of norepinephrine. Mass spectral analysis indicated the presence of a second trimethylsilyl group in the molecule, probably on the carbamate nitrogen. No characteristic fragments were observed, but the shorter retention time on a polar stationary phase and similar reports in the literature on the pertrimethylsilylation of catecholamines[1–3] strongly support this hypothesis.

Relatively mild silylation conditions with a mixture of hexamethyldisilazane and trimethylchlorosilane at room temperature were therefore used. Single peaks were obtained with the chloroformate derivatives of norepinephrine. A reaction time of 60 min was adequate for the formation of trimethylsilyl derivatives of epinephrine and norepinephrine, whereas the N-n-hexylnorepinephrine derivative required 90 min.

Stability of the chloroformate derivatives

Chloroformate derivatives. The methyl chloroformate derivatives were stable for at least 5 min in the system methylene chloride–phosphate buffer (pH 7.4, 5 or 2). The derivatives were also stable for up to 90 min in the reaction mixture before extraction with methylene chloride. The final pH was 6.4.

The alcohol group. No decrease in the yield of the methyl chloroformate derivatives resulting from derivatization of the alcohol was observed, although alcohols have been reported to react with chloroformates[27]. This was confirmed when the derivatives were allowed to stand for 1 h at room temperature with 20 μl of methyl chloroformate.

Trimethylsilylated chloroformate derivatives. The stability of the trimethyl-silylated methyl chloroformate derivatives in an ethyl acetate–buffer system was investigated. The data in Table VI indicate that some degradation took place upon prolonged standing after equilibration with acidic aqueous phase. This was especially true with norepinephrine.

TABLE VI

STABILITY OF TRIMETHYLSILYLATED CATECHOLAMINE METHYL CHLORO-FORMATE DERIVATIVES

Organic phase: 0.5 ml of ethyl acetate containing 64 μg/ml of epinephrine and 118 μg/ml of nor-epinephrine, both fully derivatized with methyl chloroformate and trimethylsilylated. Amount of internal standard: 16 μg/ml. Aqueous phase: 0.5 ml of 0.1 M phosphoric acid, phosphate buffer (pH 5 or 7.4) (μ = 1). The phases were shaken for 60 sec and allowed to stand for 90 and 180 min before analysis by gas chromatography with the NP detector.

pH of aqueous phase	% Remaining			
	Epinephrine		Norepinephrine	
	90 min	180 min	90 min	180 min
2	98	85	97	67
	96	87	89	67
5.0	97	98	96	89
	97	100	97	90
7.4	95	100	95	100
	96	100	95	100

Solutions of the trimethylsilylated methyl chloroformate derivatives in ethyl acetate with the trichloroethyl carbamate of dibenzylamine as internal standard were found to be stable for several weeks at room temperature.

Gas chromatographic properties

As discussed above, single peaks were normally obtained after trimethyl-silylation. If OV-17 was used as stationary phase and microgram amounts of the derivatives were injected, a plateau was observed before the peaks. This phenomenon was eliminated by using OV-225 or QF-1 as stationary phase. The effect was also noted to some extent on these two phases when 100-ng amounts were injected on the column and the NP detector was used. It could be reduced, although not completely eliminated, by treating new columns with Silyl-8. The phenomenon seems to be due to decomposition of the phenolic moiety of the moelcule, as the effect was less pronounced with the more bulky isobutyl chloroformate derivatives than with corresponding methyl derivatives and also less apparent with catecholamine metabolites in which one phenol group was blocked (*e.g.*, metanephrine). The superiority of isobutyl over *n*-alkyl chloroformates has been observed for the methyl ester of tyrosine[33]. Thermal decomposition in the injector can be ruled out, as no change was found when the temperature of the injector was varied between 200° and 350°. The effect is probably due to the support and the contact time with it, as it seems that the important parameter is the residence time in the column. The area of the plateau was proportional to the retention time, independent of whether the latter had

been varied by changing the carrier gas flow-rate or the oven temperature. Amino acid derivatives have been the subject of similar studies[34].

The column packings used with good results were 3% QF-1 on Gas-Chrom Q or Chromosorb W HP or OV-210 on Super Pak 20 M (Carbowax deactivated support). OV-225 could not be evaluated with the NP detector when nanogram amounts were injected.

The relative retention values of the derivatives on 3% QF-1 on Chromosorb W HP are given in Table VII. No chromatographic interference from metabolites is likely. A markedly different selectivity was found when Super Pak 20 M was used as support (Table VIII). The norepinephrine derivatives are more retarded relative to the epinephrine derivatives than when Chromosorb W HP is used.

TABLE VII

RELATIVE RETENTION TIMES OF CATECHOLAMINE DERIVATIVES: POSSIBLE INTERFERENCE FROM RELATED COMPOUNDS AND METABOLITES

3% QF-1 on Chromosorb W HP (80–100 mesh).

Compound	Relative retention time
Trichloroethyl carbamate of dibenzylamine (internal standard)	1.00*
Methyl chloroformate derivatives:	
Tyramine	0.65
Normetanephrine	1.25
Epinephrine	2.15
Dihydroxybenzylamine	2.20
Norepinephrine	2.55
N-n-Butylnorepinephrine	2.74
N-n-Hexylnorepinephrine	4.08
Dopamine	2.82

* Absolute retention time: 1 min at 220°.

TABLE VIII

RELATIVE RETENTION TIMES OF CATECHOLAMINE DERIVATIVES: SELECTIVITY OF DIFFERENT SUPPORTS

Compound	Derivative	Relative retention time	
		3% QF-1 on Chromosorb W HP	3% OV-210 on Super Pak 20 M
Epinephrine	Methyl chloroformate	1.00	1.00
Norepinephrine	Methyl chloroformate	1.19	1.60
N-n-Hexylnorepinephrine	Methyl chloroformate	1.90	2.25
Epinephrine	Ethyl chloroformate	1.00	1.00
Norepinephrine	Ethyl chloroformate	1.23	1.47
N-n-Hexylnorepinephrine	Ethyl chloroformate	1.73	1.67
Epinephrine	Acetyl	1.00	1.00
Norepinephrine	Acetyl	1.02	1.37
Epinephrine	Propionyl	1.00	1.00
Norepinephrine	Propionyl	1.19	1.40

The use of ethyl or isobutyl chloroformate increase the retention time, the latter being retained almost four times as long as the corresponding methyl derivatives. However, the temperature required for elution within 5 min is moderate (230°, gas chromatography–mass spectrometry).

Quantitative determinations with the NP detector

Methyl chloroformate derivatization of epinephrine and norepinephrine was used in quantitation in aqueous solutions at levels down to 60 and 200 ng/ml, respectively, with a relative standard deviation of 2.4% ($n=10$) of the measured peak-height ratio. The corresponding value when analysing 1.0 ml of plasma was 5.6%. The difference in the detectability of the catecholamine derivatives is probably a result of their differing ability to form CN radicals in the detector[35].

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