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Gas Chromatography of Catecholamines as Their Trifluoroacetates

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Catecholamines were treated with trifluoroacetic anhydride in tetrahydrofuran at a room temperature. The reaction mixture was diluted with *n*-hexane and was injected directly onto a gas chromatograph. A complete separation of three catecholamines, epinephrine, dopamine and norepinephrine, was achieved on a 2% GE-XF 1105 glass column at 190°. Isodrin was used as an internal standard. The formation of the trifluoroacetates was rapid and simple. The trifluoroacetates produced were volatile enough to permit the use of selective columns, and satisfactory responses were obtained from several ng of them using an electron capture detector.

Gas chromatographic studies on the catecholamines have been conducted by numerous investigators,[2-6] although none of them succeeded in a separation of the catecholamines in biological materials. Recently we achieved the separation of the catecholamines in bovine adrenal medulla by conversion of the hydroxyl groups to their trimethylsilyl ethers through a reaction with hexamethyldisilazane in a dimethyl sulfoxide, followed by a condensation with ketones.[7,8] However, owing to the limited sensitivity of hydrogen flame ionization detector, the method is still unapplicable to materials of low concentrations of catecholamines such as urine. Horning, *et al.*[4] suggested the usefulness of the pentafluoropropionylation for the estimation of very small amounts of catecholamines using electron capture detector. In the present work, gas chromatographic analysis of minute amounts of catecholamines as their trifluoacetyl derivatives were investigated.

Experimental

Apparatus and Condition——A Shimadzu Model GC-1C gas chromatograph equipped with an electron capture detector and glass tubes for columns, 2.0 m in length and 4 mm in internal diameter was used. The column packings were 2% GE-XF 1105, 12% DC 1107 and 7% DC 560 on Gas-Chrom P (80—100 mesh). The gas chromatography parameters in case of 2% GE-XF 1105 were as follows: Column (and injection) temperature, 190°; detector temperature, 200°; nitrogen flow rate, about 80 ml/min; sensitivity, 100; range, 1.6; applied voltage, 10 V.

Preparation of Trifluoroacetyl Derivatives——One ml of a sample solution in 0.1 N AcOH containing about 1 μg of each catecholamine was evaporated to dryness in a water bath of 50° at a reduced pressure. The residue was treated with 0.05 ml of tetrahydrofuran and one drop of trifluoroacetic anhydride for 10 min at a room temperature. The reaction mixture was diluted to an appropriate volume with *n*-hexane and was injected directly to a gas chromatograph.

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Results and Discussion

Trifluoroacetylation of catecholamines proceeded rapidly without the need of catalyst at a room temperature and was accomplished in several minutes. The trifluoroacetates produced were volatile enough to permit the use of selective columns at a relatively low temperature.

Relative retention times for trifluoroacetyl derivatives of catecholamines and related compounds are shown in Table I including several compounds expected as internal standards.

A satisfactory separation of three catecholamines, epinephrine, dopamine and norepinephrine, was readily achieved on GE–XF 1105 (nitril silicone) column as illustrated in Fig. 1 together with isodrin as an internal standard.

Table I. Relative Retention Times of Trifluoroacetates of Catecholamines and Related Compounds, and of Several Compounds expected as Internal Standards

Compounds	2% GE–XF1105 190°	12% DC1107 170°	7% DC560 150°
Benzophenone	0.66		
Tyramine TFA[a)]	0.69	0.87	0.80
Epinephrine TFA	1.00	1.00	1.00
Octopamine TFA	1.10	0.84	0.76
Metanephrine TFA	1.23	1.65	1.71
Dopamine TFA	1.38	1.19	1.15
Norepinephrine TFA	1.92	0.92	0.86
Normetanephrine TFA	1.96	1.38	1.40
Isodrin	2.48		
Allethrin	3.73		

a) TFA: trifluoroacetyl derivative

As would be expected, trifluoroacetates of the catecholamines showed excellent electron capture properties, and satisfactory responses were obtained from 1—2 ng of the amines. The reagent trifluoroacetic anhydride was also highly senstivie for an electron capture detector, so its amount used should be reduced as possible. As reaction media, acetonitril, pyridine, tetrahydrofuran and *n*-hexane were tested and tetrahydrofuran was found to be most suitable. Pyridine reacted vigorously with the reagent and the solution colored yellow.

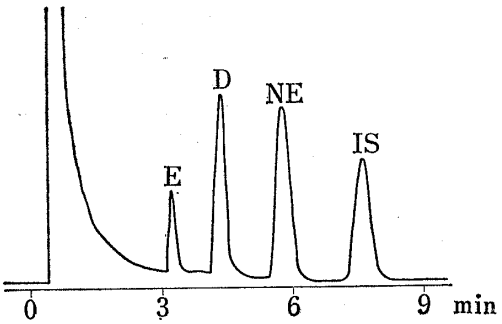

Fig. 1. Separation of Three Catecholamines as the Trifluoroacetates

E: epinephrine, D: dopamine, NE: norepinephrine
IS: internal standard (isodrin)
Condition: 2%–GE–XF 1105 on Gas-ChromP (80—100 mesh); glass column, 2.0 m × 4 mm; 190° and about 80 ml of nitrogen per min.
Detector: an electron capture detector

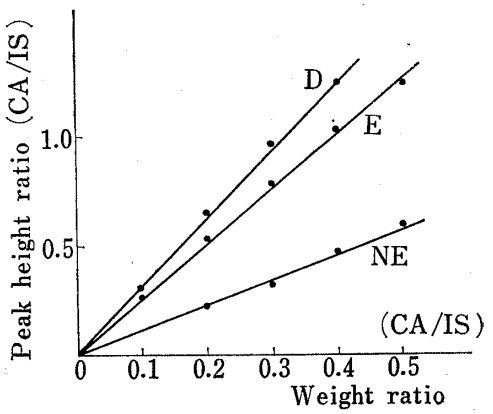

Fig. 2. Calibration Curves for Epinephrine (E), Dopamine (D), and Norepinephrine (NE) using Isodrin as an Internal Standrad

n–Hexane was unmiscible with the reagent. Acetonitril gave a longer tail than tetrahydrofuran on the gas chromatogram. When an electron capture detector was used, a glass column was superior to a stainless one in which a satisfactory peak could not be obtained. Table II shows the variation of the data obtained from gas chromatographic analysis of epinephrine, in which the standard deviation was about 4%.

Table II. Variation of the Data in Gas Chromatographic Analysis[a] of Epinephrine[b]

	Peak height of benzophenone (B) (mm)	Peak height of epinephrine (E) (mm)	E/B
1)	113.2	133.0	1.18
2)	117.2	132.1	1.13
3)	113.5	138.0	1.22
4)	123.0	145.0	1.18
5)	121.0	146.0	1.21
Average			1.18
Standard deviation			0.04

a) 2% GE–XF1105 glass column, 2.0 m, 190°, electron capture detector.
b) After evaporation and trifluoroacetylation of 20 μg of epinephrine in 1.0 ml of 0.1 N acetic acid, 30 μg of benzophenone in 5 ml of *n*–hexane was added and 1 μl of the solution was injected onto the gas chromatograph.

Horning[4] reported the erratic results caused by the direct injection of reaction mixture containing pentafluoropropionic acid and pyridine, while in our case using trifluoroacetic anhydride without pyridine, no disturbance was observed. Fig. 2 shows calibration curves of epinephrine, dopamine and norepinephrine using isodrin as an internal standard. As the peak height ratio of two compounds varied with varying of the amounts injected into a gas chromatograph, attention was paid so that an amount of an internal standard injected was as constant as possible.

The gas chromatography of the catecholamines as their trifluoroacetates using an electron capture detector is sensitive enough for their determination in urine and it seems to be superior to fluorometric method as regards selectivity. Applications of the method to the analysis of catecholamines in urine and tumor will be reported in the following paper.[9]

9) S. Kawai and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), in press.