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Note

The gas–liquid chromatographic separation of selected catecholamines on polyamide A103

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The use of polyamide A103 (Poly A103) phase for gas–liquid chromatographic (GLC) analysis of standard catecholamine solutions has been reported using mixed trifluoroacetyl–trimethylsilyl (TFA–TMS) derivatives[1]. In the present study this method of analysis has been applied to human urine for identification of conjugated dopamine (DA), tyramine (TYR), normetanephrine (NM) and metanephrine (MN). An additional method for catecholamine analysis employing trimethylsilyl (TMS) derivatives of NM, MN, DA, TYR, and norepinephrine (NE) on Poly A103 is presented.

EXPERIMENTAL

Preparation of TMS derivatives of DA, TYR, MN, NM, NE

Aliquots (100–500 μmoles) of each catecholamine solution, prepared in 0.001 *N* HCl, were dried in a Virtis Bio-Dryer. After flushing the vials with nitrogen, 50 μl of BSA [N,O-bis(trimethylsilyl)acetamide] (Applied Science Lab., State College, Pa., U.S.A.) was injected through a PTFE tape cover, screw caps were put in place, and the vials were heated at 76° for 3–4 h. Aliquots of the TMS derivative solutions were injected directly on the gas chromatograph.

Preparation of TFA–TMS derivatives of DA, TYR, NM, MN, NE

Solutions of TYR (14 μmoles), DA (12 μmoles), MN (109 μmoles), NM (109 μmoles), NE (102 μmoles) were prepared in 0.001 *N* HCl and brought to dryness under vacuum (Virtis Bio-Dryer). Mixed TFA–TMS derivatives were prepared by a modification of the method of Cancalon and Klingman[1]. Each standard catecholamine solution was dissolved in 50 μl methylene chloride and 50 μl of trifluoroacetic anhydride (Eastman, Rochester, N.Y., U.S.A.) was added. The reaction time was 7 min at 140°. After cooling and drying, 50 μl of pyridine and 20 μl of BSTFA [bis (trimethylsilyl)trifluoroacetamide] (Supelco, Bellefonte, Pa., U.S.A.) were added. The vials were heated at 105° for 25 min, cooled in ice and reduced in volume in the Bio-Dryer. Acetone was added to bring the final volume of solution to 7–8 μl and the entire solution was injected on the gas chromatograph.

Preparation of urinary NM, MN, DA and TYR derivatives

Conjugated NM, MN, DA and TYR were isolated from human urine using sequential Dowex 50-X4 (H^+) columns. An aliquot of urine was applied to the first column, the water effluent was collected, hydrolyzed at pH 1.0, and applied to a second column. MN, NM, and DA were eluted with 1 *N* and TYR with 2 *N* HCl. The eluates were lyophilized and derivatives prepared as described above except that 50 μl of pyridine was the solvent for the BSA reaction.

Gas–liquid chromatography

All GLC analyses were performed on glass columns (4 ft. × 4 mm I.D.) containing 3% Poly A103 on 100–120 mesh Gas-Chrom Q (Applied Science Lab.). The TMS derivatives were separated isothermally at 160°, 170° and 180° as well as by temperature programming from 150° to 200° at a rate of 4°/min. The mixed (TFA–TMS) derivatives were separated isothermally at 180° and 200° in addition to the temperature program of Cancalon and Klingman[1], 75–250° at 4°/min. The following conditions were employed for these studies: injection port, 230°; flame ionization detector, 250°; carrier gas (helium) flow-rate, 60 ml/min.

RESULTS AND DISCUSSION

Table I presents the chromatographic separation of the TMS derivatives prepared by reaction with BSA. No attempt was made to prepare quantitatively these derivatives and in fact, other derivatives may be seen in low concentration[2]. The complete separation of MN and NM was not achieved by either the isothermal or the temperature program conditions tested. Isothermally the overall separation of the catecholamines was best accomplished at 170° (Fig. 1), without improvement at either 160° or 180°. The temperature programmed chromatogram resembled that obtained isothermally at 170°. Low concentrations of MN and NM on both systems gave a double peak with NM preceding MN.

TABLE I

GAS CHROMATOGRAPHIC SEPARATION OF THE TMS DERIVATIVES OF CATECHOLAMINES ON POLY A103

Derivative	*Relative retention time, isothermal at 180°*	*Temperature program (4°/min)*
NE	0.45	168°
NM	0.51	170°
MN	0.54	170°
TYR	0.68	180°
DA	1.00	190°

The separation of urinary NE, MN, NM and DA by Dowex-50 column chromatography has been reported[3]. In the present study TYR was eluted with 2 *N* HCl. The BSA-prepared TMS derivatives of the appropriate fractions of TYR, MN and NM, and DA have been successfully chromatographed by temperature programming (150–200°) on Poly A103.

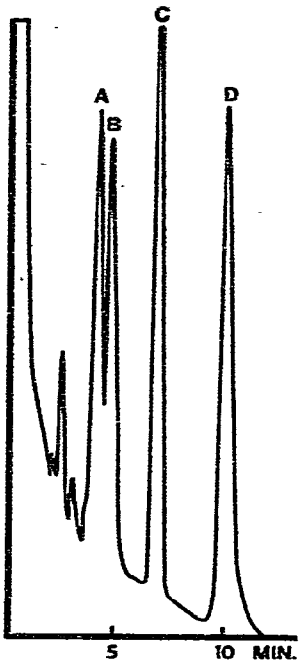

Fig. 1. Gas–liquid chromatogram on Poly A103 of the BSA prepared TMS derivatives of (A) norepinephrine, (B) metanephrine and normetanephrine, (C) tyramine, and (D) dopamine. Isothermal conditions were employed at 170°.

The mixed TFA–TMS derivatives of DA, TYR and NE have been reported by Cancalon and Klingman[1]. In the current study the positions of MN and NM at 190 and 202°, respectively, (Table II) are added to the temperature programmed chromatogram. In addition, the isothermal (180°) separation of the mixed TFA–TMS derivatives of MN, TYR, NM and DA on Poly A103 is here reported. Difficulty was encountered with the formation of the mixed derivative of NE. Two peaks were found on the temperature programmed chromatogram at 170° and 190°; the previously reported[1] NE derivative should elute at 212°. The NM derivative also gave a second peak at 170°. In both cases the two peaks were also detected by the isothermal GLC system. The mixed derivative of urinary DA was readily chromatographed by temperature programming (Fig. 2). By comparison with the standard catecholamine

TABLE II

GAS CHROMATOGRAPHIC SEPARATION OF THE TFA–TMS DERIVATIVES OF CATECHOLAMINES ON POLY A103

Derivative	*Relative retention time, isothermal at 180°*	*Temperature program (4°/min)*
MN	0.43	190°
TYR	0.65	200°
NM	0.71	202°
DA	1.00	210°

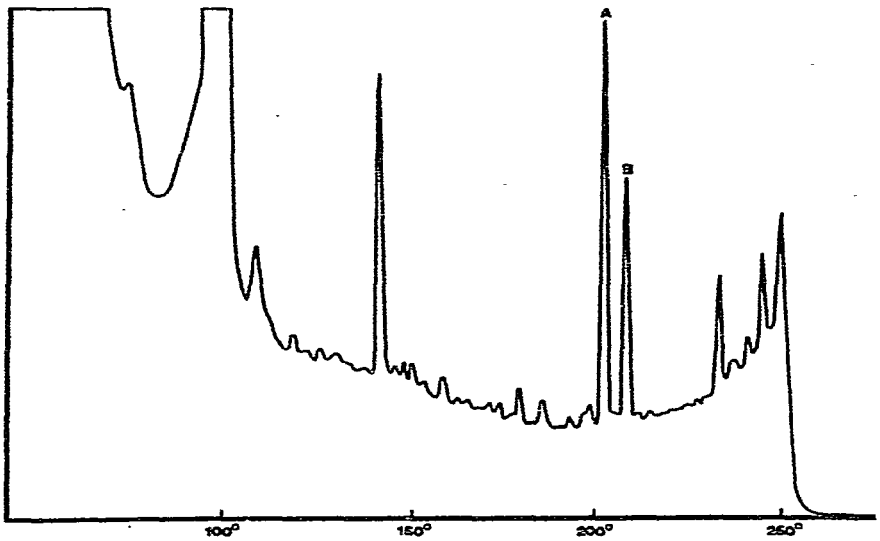

Fig. 2. Gas–liquid chromatogram on Poly A103 of an eluate from a Dowex-50 column of human urine. Dopamine (B) is separated from an unknown component (A) by use of temperature programming of the mixed TFA–TMS derivative.

elution temperatures (Table II) the peak preceding DA is neither MN nor NM, thereby demonstrating the complete separation of MN and NM from DA on the Dowex column. The mixed derivative of urinary TYR was similarly chromatographed with virtually no additional components seen.

It is concluded that Poly A103 is a useful phase for the GLC analysis of NE, NM and MN, TYR and DA utilizing either the TMS or TFA–TMS derivatives. This system has been applied to the study of urinary catecholamines.

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