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Note

Chromatographic separation of catecholamines on a weakly acidic ion-exchange resin using a borate-containing eluent

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It has been reported that catecholamines adsorbed on a column of Amberlite IRC-50 buffered at pH 6.0–6.5 could be eluted with aqueous boric acid solution[1–4]. Separation of norepinephrine and dopamine has been achieved by using 0.7% aqueous boric acid solution as the eluent[4].

In the present investigation, the chromatographic separation of catecholamines on a buffered Amberlite IRC-50 column was studied using a boric acid-containing buffer as eluent.

EXPERIMENTAL

Materials

Epinephrine hydrogen tartrate was purchased from Nakarai (Kyoto, Japan) and norepinephrine hydrogen tartrate, isoproterenol hydrochloride, dopamine hydrochloride and deoxyepinephrine hydrochloride from Yashima (Osaka, Japan); other chemicals were of reagent grade. Stock solutions of catecholamines corresponding to 1 mg/ml of the catecholamine base were prepared in 0.01 *M* hydrochloric acid.

Ion-exchange resin

Amberlite IRC-50 (A.G.) was pulverized, graded according to size and washed as described previously[5]; the fraction of size range 50–65 μm in the wet sodium ion form was used. A suspension of the washed resin in the sodium ion form was buffered at the pH of the eluent with phosphate–boric acid solution 1 or 3 (Table I). Solution 1 was used when the eluent to be used contained 1/6 *M* boric acid and solution 3 was used when the eluent to be used contained 2/3 *M* boric acid. Eluents were prepared by mixing phosphate–boric acid solutions as indicated in Table II.

Preparation of columns

After being washed with the eluent to be used for the chromatographic separation, the thick suspension of the resin was poured into a column and allowed to settle under gravity. The column was then washed overnight with the eluent under a hydrostatic pressure of 0.2–0.25 kg/cm^2 at 30°.

TABLE I

COMPOSITIONS OF PHOSPHATE–BORIC ACID SOLUTIONS

Solution	*Concentration of constituents (M)*				
	Boric acid	NaH_2PO_4	Na_2HPO_4	$EDTANa_2$*	*NaCl*
1	1/6	0.10		0.005	
2	1/6		0.05	0.005	
3	2/3	0.10		0.005	
4	2/3	0.10		0.005	0.11
5	2/3		0.05	0.005	0.11

* Ethylenediaminetetraacetic acid disodium salt.

TABLE II

COMPOSITIONS OF ELUENTS

Eluent	*pH**	*Solutions to be mixed***
A	6.60	1 and 2
B	6.00	4 and 5

* pH was measured at 20° using a Model HM-5A glass electrode pH meter manufactured by TOA Electronics (Tokyo, Japan).

** Phosphate–boric acid solutions listed in Table I.

TABLE III

CONDITIONS FOR CHROMATOGRAPHIC SEPARATION ON AMBERLITE IRC-50

In all instances, the column diameter was 9 mm.

Figure	*Eluent*	*Column length (cm)*	*Flow-rate (ml/h)*	*Mean volume of one fraction (ml)*
1	A	42.0	14.8	1.48
2	B	59.5	11.9	0.99

Chromatographic separation of synthetic mixtures

The conditions for the chromatographic separation are given in Table III. Stock solutions of each amine were mixed and diluted with the eluent to give an amine base concentration of 1–5 μg/ml. A 2.0-ml volume of the solution was added to the column. After the solution had drained into the column, the catecholamines were eluted at 30° under a hydrostatic pressure of 0.2–0.25 kg/cm² and the eluate was collected using a timer-operated fraction collector. Each fraction was mixed with 2.0 ml of 0.3 *M* hydrochloric acid and the fluorescence was measured at 315 nm, with excitation at 280 nm, by using a Hitachi Model MPF-2A fluorescence spectrophotometer.

RESULTS AND DISCUSSION

It was found that elution of catecholamines from a column of buffered Amberlite IRC-50 was governed mainly by pH, boric acid concentration and sodium

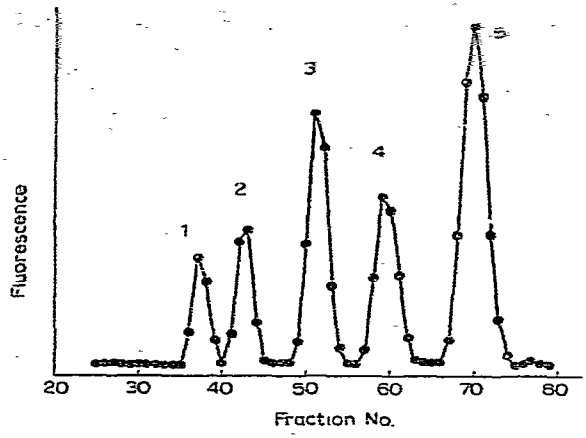

Fig. 1. Elution of catecholamines with eluent A under the conditions given in Table III. Peaks: 1 = isoproterenol; 2 = epinephrine; 3 = norepinephrine; 4 = deoxyepinephrine; 5 = dopamine.

ion concentration of the eluent, and complete separation of catecholamines was possible at pH 6.6, with eluent A containing 1/6 *M* boric acid and 0.11 *M* sodium ion (Fig. 1). With an eluent of higher pH, the elution volume of catecholamines descreased and the elution of deoxyepinephrine and dopamine was accelerated more than that of the other three catecholamines, resulting in overlap of deoxyepinephrine and norepinephrine at pH 7.0. With an eluent of lower pH, the elution volume of catecholamines increased and the elution of deoxyepinephrine and dopamine was retarded more than that of norepinephrine, but the separation of isoproterenol, epinephrine and norepinephrine was not improved.

An increase in the boric acid concentration of the eluent resulted in a decrease of the elution volume of catecholamines. With the eluent of pH 6.6 containing 2/3 *M* boric acid and 0.11 *M* sodium ion, catecholamines were eluted closer to each other, and deoxyepinephrine overlapped with norepinephrine. The separation of deoxy-

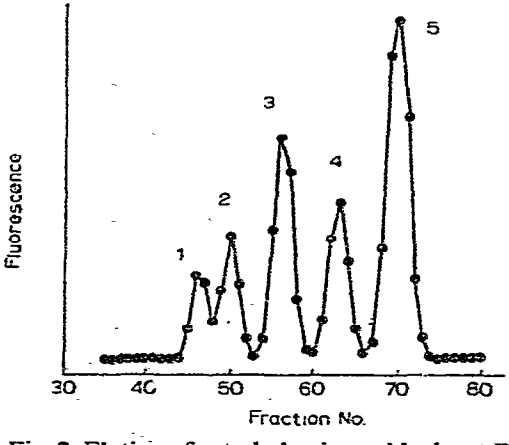

Fig. 2. Elution of catecholamines with eluent B under the conditions given in Table III. Peaks: 1 = isoproterenol; 2 = epinephrine; 3 = norepinephrine; 4 = deoxyepinephrine; 5 = dopamine.

TABLE IV

RECOVERY OF CATECHOLAMINES FROM THE COLUMN

Catecholamine	*Eluent A*		*Eluent B*	
	Added (μg)	*Recovered (μg)*	*Added (μg)*	*Recovered (μg)*
Isoproterenol	2.0	2.06	2.0	1.80
Epinephrine	2.0	2.04	2.0	2.08
Norepinephrine	4.0	4.21	4.0	3.74
Deoxyepinephrine	4.0	4.11	4.0	4.07
Dopamine	10.0	9.66	10.0	9.16

epinephrine from norepinephrine could be achieved by using an eluent of lower pH, but the separation of epinephrine from norepinephrine became poor. An increase in the sodium ion concentration of an eluent was found to have a similar effect on the elution of catecholamines to an increase in the pH of the eluent, and when a mixed buffer of pH 6.0 containing 2/3 *M* boric acid was used as the eluent the optimal sodium ion concentration of the eluent for the separation of five catecholamines was 0.22 *M* (eluent B, Fig. 2).

Although a better separation of catecholamines was obtained with eluent A, eluent B containing 2/3 M boric acid will be more suitable for the analysis of the catecholamines from biologocal samples, as the catecholamine fraction eluted from a column of Amberlite CG-50 with 2/3 *M* boric acid solution can be added directly to the analytical column equilibrated with eluent B after adjusting its pH to 5.9–6.0 (refs. 2 and 6).

The column could be used repeatedly and the recovery of catecholamines from the column was satisfactory (Table IV). The application of the chromatographic system to the quantitative analysis of catecholamines in biological samples will be reported later.

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