Journal of Chromatography, 207 (1981) 286–287
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,499

Note

Chromatographic separation of some biogenic amines on a weakly acidic ion-exchange resin

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(Received November 4th, 1980)

Chromatographic separation of biogenic amines has been performed by ion-exchange chromatography[1–7], reversed-phase partition chromatography and paired-ion chromatography[8–10]. These methods provide a good separation of catecholamines and their metabolites; however, a simultaneous separation of basic metabolites of catecholamines and serotonin by means of isocratic elution has not been reported.

We have found that catecholamines can be eluted isocratically from a column of Amberlite IRC-50 with a buffer of pH 4. Various buffers were tried as eluents, and separation of catecholamines, octopamine, 3-O-methylated catecholamines and serotonin was achieved with a buffer of pH 4.4 containing propionate (0.15 *M*), tartrate (0.10 *M*), EDTA (0.002 *M*) and boric acid (0.35 *M*) as the eluent.

CHROMATOGRAPHIC SYSTEM

Amberlite IRC-50 (Na^+; particle size 50–60 μm) was buffered at pH 4.4 and washed with the eluent and packed into a chromatographic tube (0.8 cm I.D.) equipped with a column adjuster. The eluent was pumped into the column at a flow-rate of 1.0 ml/min (a constant delivery pump, Jasco Model LCP-150) at 50°C, and the final length of the column was 24 cm. The sample was dissolved in the eluent and 1.0 ml of the solution was added to the column using a loop injector (Kyowa Seimitsu, sampler, Model M2). Amines in the eluate were monitored fluorometrically (spectrofluorometer, Jasco Model FP-4) with excitation at 285 nm and emission at 325 nm.

RESULTS AND DISCUSSION

The elution pattern is shown in Fig. 1. Amines were eluted in the order of decreasing polarity. Propionic acid was incorporated in the eluent in order to reduce non-ionic adsorption of amines on the resin. Separation of dopamine and octopamine was possible only when both tartrate and boric acid were present in the eluent. Reduction of retention time of catecholamines by the use of the eluent seemed to be due to the formation of a negatively charged catecholamine–borate–tartrate complex at pH 4.4. At higher pH, separation of dopamine from octapamine could be improved, but separation of norepinephrine from epinephrine became worse. The elution pattern was quite reproducible and the column could be used repeatedly.

0021-9673/81/0000–0000/$02.50

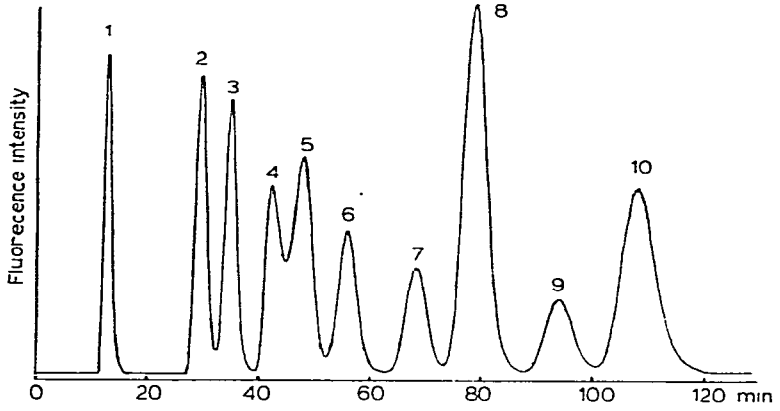

Fig. 1. Elution of standard samples of various amines. Peaks: 1 = dopa; 2 = norepinephrine; 3 = epinephrine; 4 = dopamine; 5 = octopamine; 6 = normetanephrine; 7 = metanephrine; 8 = tyramine; 9 = 3-methoxytryamine; 10 = serotonin. Column size, 24 × 0.8 cm I.D.; column temperature, 50°C; flow-rate of the mobile phase, 1.0 ml/min.

ACKNOWLEDGEMENTS

The author thanks Japan Spectroscopic Company for the loan of equipment and Atsuko Naoi for her skillful technical assistance.

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