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Note

Multi-parallel detection in high-performance liquid chromatography

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High-performance liquid chromatography (HPLC) is now widely used in many fields, including routine work such as clinical tests, but the selectivity of the detection method is sometimes poor. Also, in the analysis of a series of metabolites with different chemical characteristics, it is desirable to have a detection method involving several appropriate procedures in a single HPLC run.

In this paper we describe a multi-parallel detection method, based on the separation of the flow from the HPLC column by a simple flow separator with pressure coils.

EXPERIMENTAL

Chemicals

Catecholamines were obtained from Sigma (St. Louis, MO, U.S.A.) and other chemicals from Yoneyama Pharmaceutical (Osaka, Japan). All were of guaranteed grade and were used without further purifications.

Flow separator

The flow separator was made of a stainless-steel multi-way joint (Kyowa Seimitsu, Tokyo, Japan) and pressure coils (1 m × 0.1 mm or 2.5 m × 0.25 mm PTFE or stainless-steel tubing).

HPLC system

The HPLC system used was a TSK 805 (Toyo Soda, Tokyo, Japan), Catecholamines were separated on a 7.5 × 7.5 mm I.D. column of TSK LS 410 (ODS-type resin, 5 μm; Toyo Soda) using 0.1 *M* potassium dihydrogen orthophosphate (pH 3.1) at 25°C at a flow-rate of 1.0 ml/min. All of the catecholamines were eluted within 10 min and were then subjected to different detection methods.

Sample preparation

Sample of catecholamines from biological materials were prepared by the alumina method with dihydroxybenzylamine (DHBA) as the internal standard. For example, 70 mg of neutral alumina were used to adsorb catecholamines at pH 8.6 from 3 ml of human urine; they were subsequently eluted with 0.2 ml of 1 *M* acetic acid by the column method, followed by washing with 5 ml of 0.1 *M* potassium

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dihydrogen orthophosphate (pH 8.6) and with 0.5 ml of water. The catecholamine fraction was analysed directly by HPLC.

Detection methods

In the *o*-phthalaldehyde (OPA) method, the reagent (according to Benson and Hare[1]) was mixed with an equal volume of the column eluate. The reaction time was 20 sec for 70°C. The fluorometer used was an FLD-1 (Shimadzu, Kyoto, Japan) with a back-pressure of 1 kg/cm^2 to prevent air-bubble formation. In the trihydroxyindole (THI) method[2], the flow reaction system with air segmentation was a PRR-2[3] (Shimadzu) with an RF 500 fluorometer (Shimadzu, Japan). In the electrochemical method[4], an EC-8 electrochemical detector (Toyo Soda) was used. The working electrode was glassy carbon and the applied voltage was 0.8 V *vs.* silver-silver chloride (0.1 *N* potassium chloride solution).

RESULTS AND DISCUSSION

Flow separator

It was important to have a good flow separator, and this was very easy to achieve. The only requirements were a low dead volume and reasonable pressure coils. Supposing that the separated flow was an aqueous solution at 0.3 ml/min, a pressure coil of 1 m × 0.1 mm of PTFE tubing correspond to a pressure barrier of about 10 kg/cm^2, and in practice this was good enough in the experiments under consideration. The volume of the pressure coil was 7.8 μl, which was negligible with respect to diffusion. A schematic diagram of the flow separator is shown in Fig. 1.

With the pressure coils with a pressure barrier of 5–10 kg/cm^2 was important to maintain the flow separation constant, but even so there were some pressure differences in each flow line (for example, ± 1 kg/cm^2) after the flow separation owing to the different detection reactions involved. Also, as shown in Fig. 1, if there was no diffusion at the flow separator then the concentration of the solute was the same

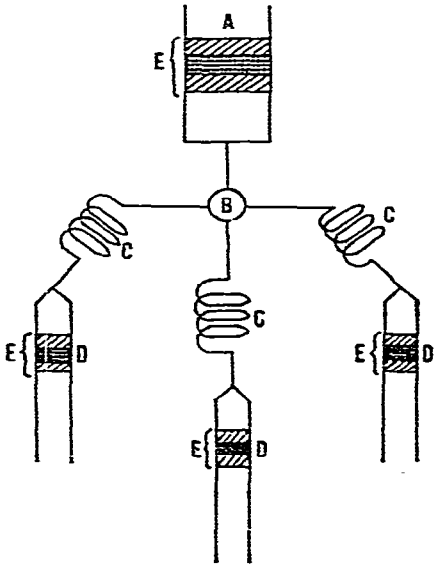

Fig. 1. Schematic diagram of the flow separator. A = HPLC separation column; B = flow separator (multi-way joint); C = pressure coils; D = pressure coils (expanded view to illustrate the concentration of the solute in the pressure coils); E = concentration of the solute in the column and in the pressure coils.

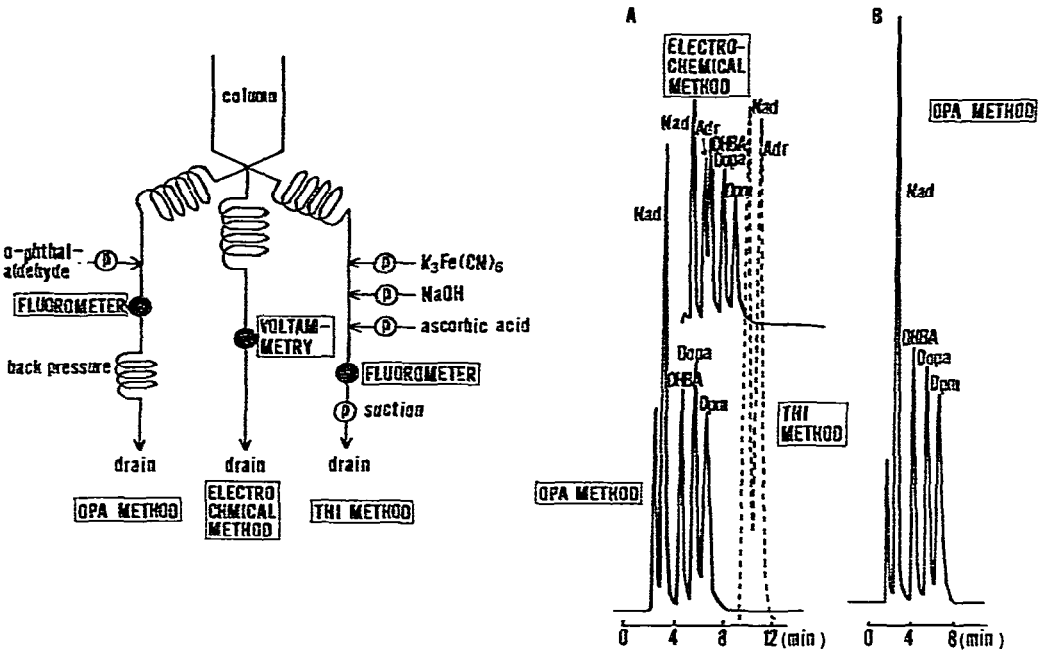

Fig. 2. Schematic diagram of the multi-parallel detection method and illustration of the detection of standard catecholamines. (A) by the multi-parallel detection method and (B) by the OPA method only (without the flow separator). The retention time of Dopa by the OPA method was 6 min. Nad = Noradrenaline; Adr = adrenaline; DHBA = dihydroxybenzylamine; Dopa = dihydroxyphenylalanine; Dpm = dopamine.

before and after the flow separator. This effect ensured that there was no loss of sensitivity in the multi-parallel detection method, as demonstrated in Fig. 2.

Standard analysis of catecholamines using the three detection methods

Catecholamines separated on the ODS-type column (TSK LS 410) were detected by the THI, OPA and electrochemical methods with the multi-parallel detection procedure as shown in Fig. 2A using a three-pen recorder. When analysing 50 pmol of each amine, the reproducibility of the peak height by the three detection methods was excellent (the coefficient of variation was less than 3%; $n = 10$). In the THI method the peaks came out later than other method because of the longer reaction time involved (*ca.* 7 min).

When the flow separator was not used, all of the eluate from the column was subjected to the OPA detection method, keeping the ratio of the eluate to the reagent constant, and the sensitivity was almost identical with that obtained in the multi-parallel detection method (Fig. 2B). The slight difference in the peak heights in Fig. 2A and B obtained with the OPA method might be due to slight differences in the reaction conditions (possibly due to a shorter reaction time). In practice, the multi-parallel detection method did not show a lower detection sensitivity, although the absolute amount to be analysed was reduced.

Analysis of catecholamine fractions from biological samples

Catecholamine fractions of rat whole brain and human obtained by the alumina method, were separated on the ODS-type column (TSK LS 410) and were detected as the above three methods (Fig. 3). Although the sample enrichment method was the same, the reliabilities of the peaks obtained by the three methods were different.

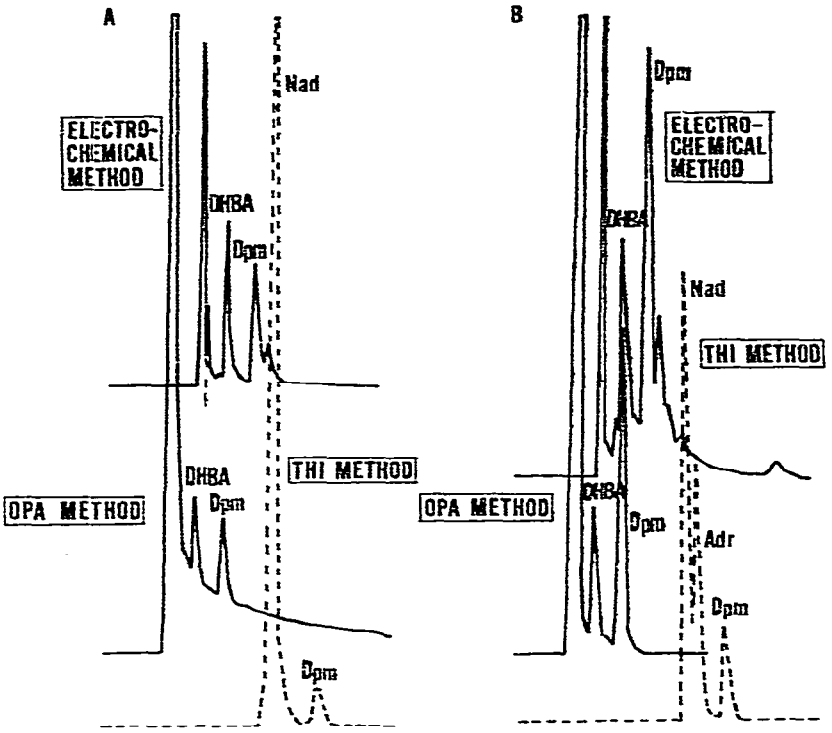

Fig. 3. Chromatogram of catecholamine fraction from (A) rat whole brain and (B) human urine by the multi-parallel detection method. The retention time of Dopa by the OPA method was 6 min. Abbreviations: see Fig. 2.

Comparison of the three methods indicates that the results obtained with the THI method were the most reliable, although another internal standard was necessary. The results obtained with the electrochemical method for rat whole brain were also reliable, but not those for human urine. The OPA method was not suitable for the analysis of either sample. The different reliabilities of the three detection methods for these samples might be due to different impurities in the catecholamine fractions.

In conclusion, the multi-parallel detection method is a useful adjunct to HPLC, and might be useful for detecting a series of metabolites that require different detection methods owing to their different chemical characteristics.

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REFERENCES

1 J. R. Benson and P. E. Hare, *Proc. Nat. Acad. Sci. U.S.*, 72 (1975) 619.
2 U. S. von Euler and I. Folding, *Acta Physiol. Scand.*, 33 Suppl. 118 (1955) 45.
3 K. Mori, *Ind. Health*, 16 (1978) 41.
4 P. T. Kissinger, C. Refshauge, R. Dreiling and R. N. Adams, *Anal. Lett.*, 6 (1973) 465.