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Note

Comparison of the sensitivity of various post-column methods for catecholamine analysis by high-performance liquid chromatography

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Recently, we reported a highly sensitive high-performance liquid chromatographic (HPLC) method for simultaneously determining picogram amounts of norepinephrine and epinephrine[1] and its application to the assay of these compounds in human plasma[2]. We have also presented a rapid and sensitive method for the determination of the regional small amounts of catecholamines in tissues[3].

In this paper, we compare the sensitivity of the various post-column methods for catecholamine analysis which are now available using HPLC.

EXPERIMENTAL

Reagents

Dopamine, norepinephrine and epinephrine were purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals were obtained from Wako (Osaka, Japan). The reagents used were of the highest grade.

Liquid chromatographic and detection methods

The following equipment, unless otherwise specified, was obtained from Shimadzu (Kyoto, Japan): LC-3A pump; SIL-1A injector, Zipax SCX column (DuPont, Wilmington, DE, U.S.A.) (1 m × 2.1 mm I.D.); PRR-2A proportioning pump; RF-500 LCA spectrofluorophotometer with 120-μl mirrored square flow cell; LC-3 amperometric detector (Bioanalytical Systems, West Lafayette, IN, U.S.A.); SPD-2A spectrophotometer; strip chart recorder (chart speed 2.5 cm/min).

Fig. 1 shows the flow scheme for electrochemical, ultraviolet and natural fluorescence detection. Samples containing dopamine, norepinephrine and epinephrine are injected through an injector onto the column for the separation with 0.15 *M* NaH_2PO_4 (pH 4.37) as the mobile phase, flow-rate 0.8 ml/min at 50 kg/cm^2. The column is operated at 40°C, and the effluent is monitored at the electrochemical detector (detector potential 0.8 V *vs.* the Ag/AgCl electrode), ultraviolet detector (at 280 nm) and spectrofluorophotometer (excitation wavelength 280 nm, emission wavelength 325 nm).

The derivatization method using *o*-phthalaldehyde is illustrated in Fig. 2. Samples are injected through an injector onto the column and eluted with 0.08 *M*

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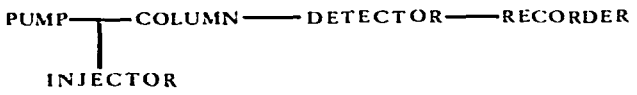

Fig. 1. Flow scheme of electrochemical, ultraviolet and natural fluorescence detection for catecholamine analysis.

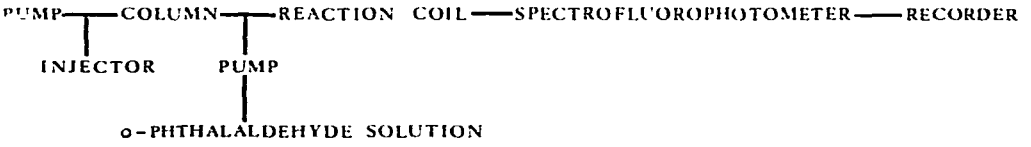

Fig. 2. Flow scheme of *o*-phthalaldehyde derivatization for catecholamine analysis.

NaH_2PO_4 as the mobile phase, flow-rate 0.5 ml/min. To the column effluent, reagents for the *o*-phthalaldehyde reaction are added at a flow-rate of 0.5 ml/min using the proportioning pump. The fluorescent products are detected at the spectrofluorophotometer (excitation wavelength 350 nm, emission wavelength 440 nm). The *o*-phthalaldehyde reagents comprise 400 mg *o*-phthalaldehyde in 7 ml ethanol and 1 ml of 2-mercaptoethanol in 500 ml boric acid solution at pH 10. The reaction (mixing) coil (2 m × 0.3 mm I.D. has 25 turns). The flow scheme and details of the trihydroxyindole reaction are as described previously[1].

RESULTS AND DISCUSSION

Table I shows the detection limits for various methods of catecholamine analysis. The electrochemical (amperometric) detection which Kissinger *et al.*[4] reported has widely been used for catecholamine analysis in tissues. However, due to its lack of specificity and sensitivity, the application to human plasma catecholamine determination has been hampered. The UV absorption method which was used in urinary catecholamine analysis[5] and the natural fluorescence detection method do not have the sensitivity and specificity for catecholamine trace analysis. To enhance the fluorescence intensity of catecholamines, some derivatizations have been employed. Fluorescamine which reacts with primary amines has most often been employed[6], and *o*-phthalaldehyde also forms a strongly fluorescent compound with primary amines in

TABLE I

COMPARISON OF THE DETECTION LIMITS OF DOPAMINE, NOREPINEPHRINE AND EPINEPHRINE

Units are pg of catecholamine.

Method	*Dopamine*	*Norepinephrine*	*Epinephrine*
1 Electrochemical	25	25	25
2 Ultraviolet	1250	1400	1200
3 Natural fluorescence	300	300	300
4 *o*-Phthalaldehyde	130	75	—
5 Trihydroxyindole	800	1	1

the presence of a reducing agent such as 2-mercaptoethanol, which is more intense than that of fluorescamine. However, these methods are less sensitive than the trihydroxyindole method and, moreover, these reagents do not react with epinephrine which is a secondary amine. Thus, for the determination of norepinephrine and epinephrine, the trihydroxyindole reaction (derivatization) seems to be the most sensitive and specific.

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