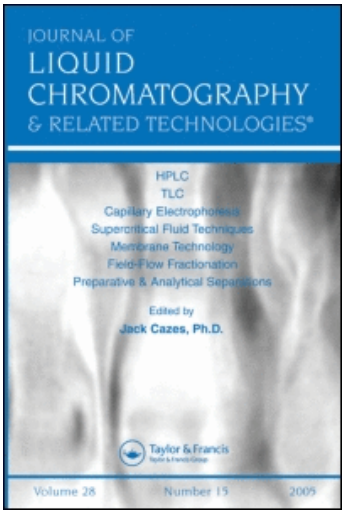

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Determination of Biogenic Amines and Their Metabolites in Regional Tissue of Mouse Brain by High Performance Liquid Chromatography Using an Ultraviolet Detector

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DETERMINATION OF BIOGENIC AMINES AND THEIR METABOLITES IN REGIONAL TISSUE OF MOUSE BRAIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING AN ULTRAVIOLET DETECTOR

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ABSTRACT

A simple method based on high-performance liquid chromatography with ultraviolet detection using octadecyl column is described for the simultaneous determination of norepinephrine, epinephrine and dopamine levels in mouse brain. Mouse brain was dissected into seven regions, striatum, hypothalamus, cerebellum, cerebral cortex, hippocampus, midbrain and medulla oblongata. The supernatant of a tissue homogenate was injected directly into a column, omitting the commonly performed adsorption step. The influence of pH, addition of acetonitrile and ion-pair reagents on chromatographic performance was studied. Assay sensitivities were sufficient for at least 20 pg of the above compounds in a mouse brain sample. The procedure is also readily applicable to determination of metabolites of catecholamines and serotonin levels.

INTRODUCTION

Changes in the activity of adrenergic nervous system have been implicated in many nutritional, physical, toxicological and

pathological states [1,2]. Three substances very important to the system are dopamine (DA), norepinephrine (NE) and epinephrine (E). Their functions are quite diverse, depending on where they are located in the organism. Procedures have been reported for catecholamine determinations based on high-performance liquid chromatography (HPLC) with either ultraviolet (UV) radiation detection [3], UV detection after sample extraction by n-butanol [4], natural fluorescence detection and fluorescence detection with post-column o-phthalaldehyde derivation [5] or dansylation for fluorescence [6], or electrochemical detection (ECD) [7-9]. However, natural fluorescence detection and UV detection are not sufficiently sensitive. Fluorescence detection by the post-column method is applicable only to primary amines and not their metabolites. Ultraviolet radiation detection and ECD are too complicated for routine analysis.

The present paper describes a method based on the simultaneous assay of NE, DA, serotonin (5-HT) and their metabolites in a single analysis. The method requires minimal sample work-up and has sufficient sensitivity for the determination of these substances in discrete brain regions. Though for ECD, the surface of electrode must be polished about once a week, using a UV detector, this method facilitates the analysis of catechol- and indoleamines, this being the most outstanding merit.

MATERIALS

Materials and their sources are as follows : 3,4-dihydroxybenzylamine (DHBA) (Aldrich, WI) ; homovanillic acid (HVA) and 3-methoxytyramine (3-MT) (Sigma, U.S.A.) : NE, E, DA and 5-HT (Tokyo Chem. Indust., Japan). One-heptane sulfonic acid (PIC B-7) and 1-octane sulfonic acid (PIC B-8) was purchased from Waters (U.S.A.).

Chromatography was performed with a Model 510 liquid chromatograph (LC) (Waters) with a Lambda-Max Model 481 LC spectrophotometer (Waters), and was equipped with a 25 cm × 4.2 mm i.d. Cosmosil 5 C_{18} column (particle size 5 μm) (Nacalai Tesque, Japan). The detector wave length was 280 nm. The mobile phase consisted of 0.1 M-citrate buffer (pH 3-5, ionic strength 0.2), acetonitrile (0-20%) and ion-pair reagents (0-15 mM). The flow rate was maintained at 1.0 ml/min and the column temperature, at 30°C by water bath.

METHODS

Male ddY strain mice (6 weeks, body weight 28.2 ± 0.7 g) were killed by decapitaion. The brains were rapidly removed according to the method of Glowinski et al. [10]. Sample preparation for chromatography was performed by the modified method of Magnusson et al. [7]. For analysis, pieces of tissue (10-50 mg) were weighed in conical 1.5 ml test-tubes. A mixed solution (PCA solution, 200-500 l) of 0.1 M perchloric acid, 4 × 10^{-5} M sodium bisulfite, and the internal standard DHBA (1 μg/ml) was pipetted into each tube, followed by sonication at 200 W for about 10 sec on ice. The homogenate was centrifuged for 2 min at 9,000 g. An aliquot of the supernatant was filtered (pore size 0.45 μm). Fifty l of filtered solution were injected into the chromatographic column.

RESULTS AND DISCUSSION

The present method was developed to assess regional levels of catechol- and indoleamines in mouse brain. Peak symmetries were satisfactory at low pH but increased with pH, slightly for

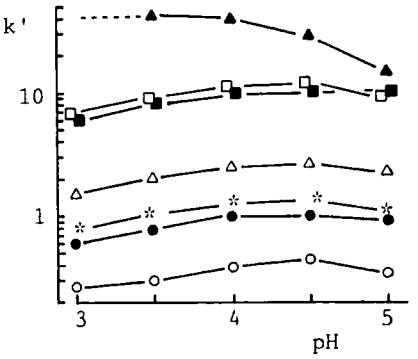

Figure 1 Effects of pH on capacity factors

Mobile phase, 0.1 M citrate buffer.
○, NE; ●, E; *, DHBA; △, DA; ▲, HVA; □, 3-MT; ■, 5-HT.

acids but greatly for the amines [7]. Consequently, the influence of low-pH on chromatography was examined. The dependence of capacity factors on pH with neat aqueous mobile phases is showed in Fig. 1. The HVA capacity factor decreased with rising pH (Fig. 1). While most of the amines separated at pH 4.5. Thus, in subsequent experiments, pH 4.5 was used.

The rather long retention times with neat aqueous buffer solutions decreased by the addition of acetonitrile. The elution order and selectivities did not change (Fig. 2). Early eluting endogenous peaks interfere with NE and E. The neat aqueous buffer solution increased the retention times of the amines by adding ion-pair reagents except HVA (Fig. 3). The addition of octane sulfonic acid to neat aqueous buffer prolonged more the capacity factors of NE and E than when heptane sulfonic acid was added. Octane sulfonic acid was thus used as the ion-pair reagent in the buffer. Separation of amines in the mobile phase (mixture of buffer, acetonitrile and octane sulfonic acid) depended primarily on the organic modifier (Fig. 4). Octane sulfonic acid was partitioned sufficiently in the column at 5 mM.

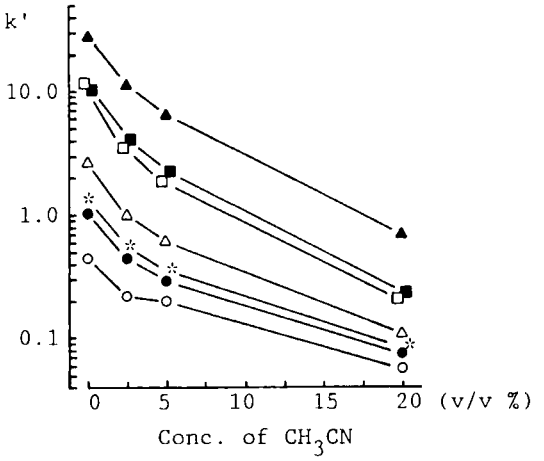

Figure 2 Effects of acetonitrile proportions on capacity factors

Mobile phase, 0.1 M citrate buffer (pH 4.5) and acetonitrile.
○, NE; ●, E; *, DHBA; △, DA; ▲, HVA; □, 3-MT; ■, 5-HT.

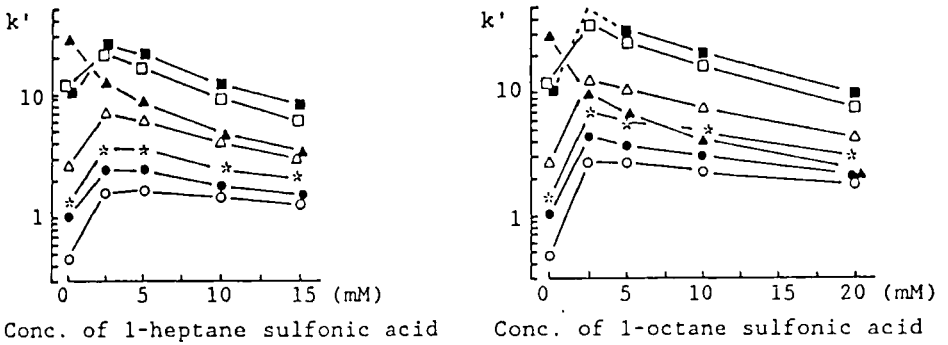

Figure 3 Effects of ion-pair reagents concentration on capacity factors

Mobile phase, 0.1 M citrate buffer (pH 4.5) and ion-pair reagent.
○, NE; ●, E, *, DHBA; △, DA; ▲, HVA; □, 3-MT; ■, 5-HT.

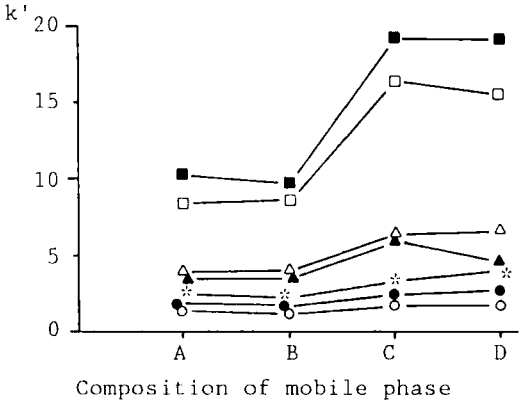

Figure 4 Effects of mobile phase composition on capacity factors

A Buffer : CH_3CN = 95 : 5 (v/v); 5 mM octane sulfonic acid.
B Buffer : CH_3CN = 95 : 5 (v/v); 2.5 mM octane sulfonic acid.
C Buffer : CH_3CN = 97.5 : 2.5 (v/v); 2.5 mM octane sulfonic acid.
D Buffer : CH_3CN = 97.5 : 2.5 (v/v); 5 mM octane sulfonic acid.
Buffer , 0.1 M citrate buffer (pH 4.5).
○, NE; ●, E; *, DHBA; △, DA; ▲, HVA; □, 3-MT; ■, 5-HT.

Calibration curves of standards in the present method were found to be linear (correlation coefficient : 0.999-0.995) from 20 pg to 1 g with NE, E, DA, HVA, 3-MT and 5-HT per tissue sample. The detection limit of the standard solution of NE and E was 20 pg, DHBA, DA, HVA, 3-MT and 5-HT was 50 pg at 0.005 AUFS.

Figure 5 shows the separation of pure standards of amines and typical chromatograms of sample extraction from the striatum, hypothalamus and midbrain. Using the PCA solution for extraction, amine recovery was approximately 87 with ± 4% relative standard deviation. The amounts of biogenic amines found in the various brain regions are given in Table 1, and are essentially the same as those reported by ECD [11-13].

The above results demonstrate the reproducibility and application of HPLC separation, based on UV detector, to

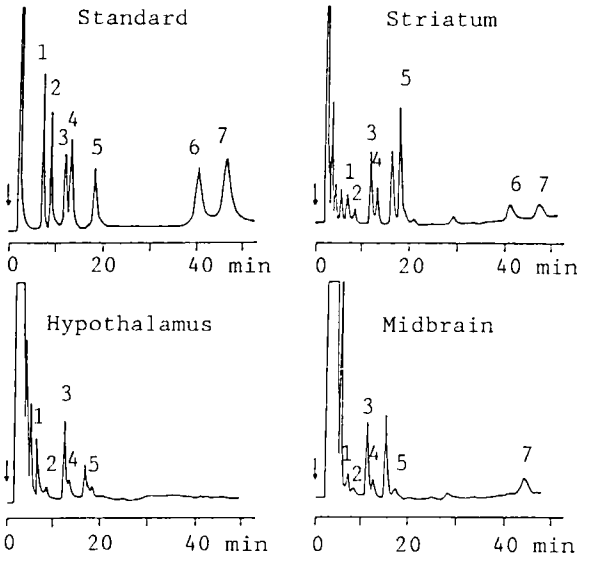

Figure 5 Chromatograms of standard slution and tissue extracts from mouse brain

Mobile phase, 0.1 M citrate buffer (pH 4.5) containing acetonitrile (2.5%) and octane sulphonic acid (5 mM). 0.005 AUFS; 4 mV per full scale. Standard solution contained 25 ng of NE, E, DHBA, HVA and DA, and 50 ng of 3-MT and 5-HT.
1, NE; 2, E; 3, DHBA; 4, DA; 5, HVA; 6, 3-MT; 7, 5-HT.

Table 1 Regional Levels of Biogenic Amines in Mouse Brain

	DA	NE	E	HVA	5-HT	3-MT
	(ng/g of wet weight)					
STR	4614±824	213± 9	88± 3	538±131	820± 99	1061±234
HT	257± 33	1647±195	78±33	153± 41	ND	ND
MB	209± 48	134± 5	53± 2	328± 19	1012± 2	ND
CC	457± 91	172± 60	33± 6	153± 22	519± 63	ND
HC	136± 9	371± 47	32± 3	ND	604± 19	ND
CER	227± 13	318± 41	48± 1	41± 2	316± 23	312± 1
MO	143± 37	148± 54	49± 5	ND	982±146	480± 39

mean ± SD, ND : not detected, n = 6.
STR, striatum; HT, hypothalamus; MB, midbrain; CC, cerebral cortex; HC, hippocampus; CER, cerebellum; MO, medulla oblongata.

measurement of major neuroactive monoamines and some of their metabolites. The adsorption procedure with alumina or ion-exchange resins is not required, greatly lessening the time for HPLC sample preparation. By the generally used UV for the detection of amines, analysis can be started quickly. Moreover, operating and maintaining the equipment is easier than ECD. Though a single analysis run including 5-HT is less than 50 min, the method is suitable for analysis of biogenic amines.

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