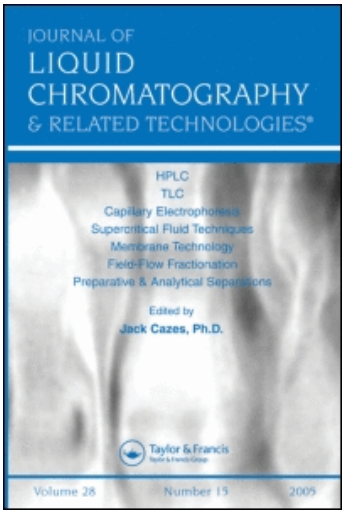

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Simultaneous Determination of Fourteen Catecholamines and Their Metabolites by High Performance Liquid Chromatography with Electrochemical Detection

J. C. Yang[a]; T. Y. Liu[a]; Y. F. Chang[a]; H. C. Liu[b]; Y. H. Shih[b]; L. S. Lee[b]; C. W. Chi[a]
[a] Department of Medical, Research Veterans General Hospital-Taipei, Taipei, Taiwan, Republic of China [b] Department of Neurological, Center Veterans General Hospital-Taipei, Taipei, Taiwan, Republic of China

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SIMULTANEOUS DETERMINATION OF FOURTEEN CATECHOLAMINES AND THEIR METABOLITES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

J. C. YANG[1], T. Y. LIU[1], Y. F. CHANG[1],
H. C. LIU[2], Y. H. SHIH[2], L. S. LEE[2], AND C. W. CHI[1]
[1]*Department of Medical Research*
[2]*Neurological Center*
Veterans General Hospital-Taipei
Taipei, Taiwan, Republic of China

ABSTRACT

A simple method has been developed for simultaneous determination of 14 catecholamines and their metabolites in cerebrospinal fluid and brain tissue by reversed-phase, ion-pair high-performance liquid chromatography with electrochemical detection. The time required for complete separation and analysis of all compounds was less than 35 min. Quantitation was based on the use of an internal standard isoproterenol. The mobile phase consisted of a 91:9 (v/v) mixture of 0.1 M formic acid and acetonitrile containing sodium-1-octane sulfonic acid. Using this method, analysis of neurotransmitters in brain tissue can be accomplished without a clean-up procedure.

Address correspondence and reprint request to :
Dr. Chin-Wen Chi, Department of Medical Research,
Veterans General Hospital-Taipei, Taipei, Taiwan 11217,
R.O.C.

The detection limits for the four compounds examined, namely 3-methoxy-4-hydroxyphenylglycol, 3,4-dihydroxyphenylacetic acid, 5-hydroxyindoleacetic acid and homovanillic acid were less than 10 pg per injection. In the cerebrospinal fluid, linear responses of the above 4 compounds were observed over the concentration range from 3.1 to 50.0 ng/ml. The coefficients of variation from 5 consecutive assays were less than 2.90% while the averaged recoveries were more than 93.37%.

INTRODUCTION

High performance liquid chromatography with electrochemical detection (HPLC-EC) techniques are now widely used for the determination of catecholamines and their metabolites in various tissues and body fluids [1-4]. However, most of the procedures presented so far measured only a few monoamine compounds simultaneously [5-11]. Few studies have been reported which permit simultaneous determination of various monoamines and their metabolites in biological tissues [8,12-16].

In order to separate a mixture of basic, neutral and acidic neurotransmitter metabolites, considerable effort in fine adjustments of the ion-pairing reagent, the organic modifier and the pH of the eluent is inevitable. In the present study, we have established an optimized isocratic chromatographic system to separate monoamines and their metabolites from other endogenous compounds in CSF and brain tissue. The method permits simultaneous analysis of fourteen catecholamine neurotransmitters in a single run of approximately 30 min without prior clean-up of the sample. The procedure is simple, sensitive and reproducible which can be easily adapted for clinical application and basic research for biological samples from the central nervous system.

MATERIALS AND METHODS

Reagents

Chemicals were obtained from the following sources : norepinephrine bitartrate (NE), dopamine HCl (DA), 3,4-dihydroxyphenylalanine (DOPA), epinephrine bitartrate (EPI), 3-methoxy-4-hydroxyphenylglycol hemipiperazine (MHPG) and 3,4-dihydroxybenzylamine HBr (DHBA) were purchased from Bioanalytical System (West Lafayetta, IN, U.S.A.). 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindole (5-HI), 5-hydroxytryptophol (5-HTOL), 3,4-dihydroxytryptamine HCl(5-HT), 5-hydroxyindoleacetic acid (5-HIAA), isoproterenol HCl (ISOP), homovanillic acid (HVA), 3-methoxytyramine HCl (3-MT), vanillylmandelic acid (VMA), normetanephrine HCl (NMN), metanephrine HCl (MN), sodium-1-octane sulfonic acid (SOS) and triethylamine (TEA) were purchased from Sigma (St. Louis, MO, U.S.A.). Reagent grade ethylenediaminetetraacetic acid (EDTA), L-ascorbic acid, citric acid, formic acid, perchloric acid (PCA) and HPLC grade acetonitrile were purchased from E. Merck (Darmstadt. Germany).

Chromatographic Conditions

The HPLC system included a Krato Spectroflow 400 pump (Ramsey NJ.), a Rheodyne 7125 sample injector fitted with a 20 µl loop and a Cosmosil (Nacalai Tesque, Japan) C18 column(4.6×250 mm, 5 µm) protected by a 7 µm Lichrosorb RP-18 (E. Merck, Germany) guard column. The system was linked to BAS LC-4B electrochemical detector (Bioanalytical System, West Lafayette, IN) with a glassy carbon electrode, and recorded on a Chromatocorder 12 integrator (System

Instruments Co., LTD. Tokyo, Japan). Detector potential was maintained at +0.75V against Ag/AgCl reference electrode, with detector sensitivity set at 20 nA/V. The entire chromatographic system was run at ambient temperature. The flow rate was 0.8 ml per minute with a back pressure of 25-30 bar.

Mobile Phase

The mobile phase was modified from the method of Hong et al. [15]. It consisted of 0.1M formic acid and acetonitrile (91:9, v/v) containing 0.56 mM SOS, 1 mM citric acid, 0.1 mM EDTA and 0.2 % (v/v) TEA. The solution was adjusted to pH 3.7 with sodium hydroxide and filtered under vacuum through a 0.45 μm Type HV filter (Millipore, Bedford, MA, USA).

Standards

Stock solutions of the reference compounds and internal standard (0.1 mg/ml) were prepared monthly in 0.1M PCA solution containing 0.134 mM EDTA and 0.01 mM ascorbic acid. Aliquots of the stock solution were stored at -20℃. Working standards (100 ng/ml) were prepared every 2 weeks from an aliquot of the stock solution by serial dilution in the same solution.

Sample Preparations and Assay

Specimens of human brain and CSF were obtained according to a protocol approved by the committe for the

conduct of human research at the Veterans General Hospital-Taipei.

Lumbar CSF (150 µl) from a patient with clinically diagnosed Alzheimer's disease and one with benign prostate hyperplasia (BPH) were precipitated with 20 µl of PEAI solution (0.1M PCA containing 0.134 mM EDTA, 0.01 mM ascorbic acid and 4 ng of ISOP as internal standard). The mixture was centrifuged at 8,500 xg for 5 min at 4℃, 20 µl of the supernatant was then injected directly into the HPLC system.

Human and rat brain tissues (60-120 mg, wet weight) were added with 400 µl of PEAI solution and homogenized with a polytron (Brinkman Instrument, NY, U.S.A.) at 4℃ for 20 sec. The homogenate was then centrifuged at 11,000 xg for 10 min at 4℃. The supernatant was filtered through a Millipore filter (pore size 0.45 µm). An aliquot (20 µl) of the filtrate was then injected into the HPLC system. For rat brain, the homogenate were diluted just before filtering and injection to minimize interference from ascorbic acid.

Levels of monoamines were determined using the peak-height ratio of sample/internal standard.

RESULTS

Linearity and Sensitivity

CSF samples were spiked with increasing amounts of catecholamines (final concentrations 3.1, 6.3, 12.5, 25.0 and 50.0 ng of each compound per ml of CSF). Calibration curves were obtained by plotting the peak height ratios of individual compounds to internal standard. Of the four compounds examined, excellent linearities were observed, with the linear regression correlation factors (r) of 0.9998,

TABLE 1

Precision of The Determination of Catecholamines and Their Metabolites in Pooled Human CSF Samples

Compound	Amount added (ng/ml)	Mean Concentration* (ng/ml)	C.V. (%)
MHPG	12.5	14.80 ±0.35	2.36
DOPAC	12.5	12.38 ±0.24	1.94
HIAA	25	35.84 ±1.04	2.90
HVA	25	31.61 ±0.70	2.21

*Values are expressed as the mean concentration ±S.D. of 5 analyses. Mean concentrations for each compound included the added and the endogenous compounds of the pooled CSF. C.V. : coefficient of variation

0.9999, 0.9996 and 0.9994 for MHPG, DOPAC, HIAA and HVA, respectively.

The detection limits of standards (amount injected, twice the noise) ranged from 2.0-5.0 pg for MHPG, 0.2-1.0 pg for DOPAC, and 8.0-10.0 pg for HIAA and HVA.

Precision and Recovery

Four compounds were used for the precision and recovery analyses, namely the MHPG, DOPAC, HIAA and HVA. The precision of our analytical procedure as determined by

TABLE 2

Analytical Recovery of Catecholamines and Their Metabolites from CSF

Compound	Amount added (ng/ml)	Mean Recovery* (%)
MHPG	12.5	93.37±2.73
DOPAC	12.5	98.16±1.88
HIAA	12.5	97.25±2.09
HVA	12.5	98.76±3.17

*Values are expressed as the mean recovery ±S.D. of 5 analyses.

making 5 consecutive injections of a pooled human CSF sample is shown in Table 1. The coefficients of variation ranged from 1.94 to 2.90%. Table 2 shows that the analytical recoveries for the four analytes ranged from 93.37 to 98.76%. Table 3 shows the exact elution times for the 14 compounds analyzed.

Fig1A shows a typical chromatogram illustrating the well separated 14 catecholamines and their metabolites. Fig1B shows the chromatogram of a lumbar CSF sample taken from a patient with BPH. Major components included MHPG, EPI, 5-HIAA and HVA. Fig1C shows the chromatogram of a CSF sample from a patient with Alzheimer's disease. The concentrations of MHPG, HIAA and HVA were lower than that of Fig1B, however, an unusual DA peak was observed. Fig1D shows the chromatographic profile of neuro-

TABLE 3

Retention Times of Catecholamines and Related Compounds

Compound		Retention time (min)
3,4-Dihydroxyphenylalanine	(DOPA)	4.61
Vanillymandelic acid	(VMA)	5.12
Norepinephrine	(NE)	6.33
3-Methoxy-4-hydroxyphenylglycol	(MHPG)	6.75
Epinephrine	(EPI)	7.34
Normetanephrine	(NMN)	9.34
Dopamine	(DA)	10.83
Metanephrine	(MN)	11.50
3,4-Dihydroxyphenylacetic acid	(DOPAC)	13.82
Isoproterenol	(ISOP)	15.92
5-Hydroxytryptophol	(5-HTOL)	18.72
3-Methoxytyramine	(3-MT)	20.97
5-Hydroxyindoleacetic acid	(5-HIAA)	23.16
5-Hydroxytryptamine	(5-HT)	24.66
Homovanillic acid	(HVA)	31.08

transmitters from a human cerebral cortex sample. Major peaks were NE, EPI, DA, DOPAC, 5-HIAA and HVA. Fig1E shows the level of neurotransmitters in a rat cerebellum tissue with 4 fold dilution. High levels of NE, DA, HIAA and 5-HT were found as compared to that of Fig1D, other minor peaks included MHPG, EPI, NMN, DOPAC, 3-MT and HVA.

DISCUSSION

In the present study, the mobile phase of our chromatographic system derived after a modification of the method of Hong et al. [15]. The original mobile phase consisted of a 91:9 (v/v) mixture of formic acid and acetonitrile with 0.35 mM SOS, and a final pH of 4.11. However, simultaneous determination of 14 compounds was not feasible in their system. By changing the concentration of ion-pairing reagent and the pH in the eluent, we have successfully achieved a chromatographic condition that permits baseline resolution and separation of 14 compounds. In addition, the inclusion of internal standard–ISOP further increased the accuracy and reliability of our method.

In this system, the addition of SOS to the mobile phase helped to get an excellent resolution of all 14 compounds. However, when the concentration of SOS was increased above 0.56 mM, the retention times were increased, and this also resulted in overlapping of DOPA and VMA, MHPG and EPI peaks as well as 5-HIAA and 5-HT peaks. In general, the addition of acetonitrile to the mobile phase has the advantage of decreasing the retention times of target compounds, lengthy peroids of separation could thus be avoided. Yet, in our chromatographic condition, when the acetonitrile concentration was raised above 10%, the

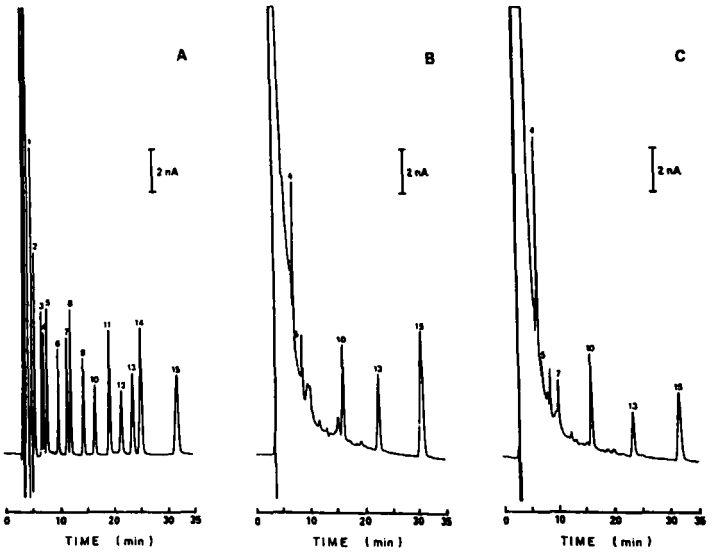

FIGURE 1. Chromatograms of (A) a standard cocktail 20 µl containing 500 pg of 5-HTOL, HIAA, 5-HT and HVA as well as 250 pg of other compounds, ISOP (470 ng) was used as internal standard, (B) a human lumbar CSF sample, (C) a CSF sample from a patient with Alzheimer's disease, (D) a human cerebral cortex tissue, (E) a rat cerebellum tissue with 4 fold dilution. Numbers in chromatograms indicate the following compounds: DOPA (1), VMA (2), NE (3), MHPG (4), EPI (5), NMN (6), DA (7), MN (8), DOPAC (9), ISOP (10), 5-HTOL (11), 3-MT (12), 5-HIAA (13), 5-HT (14), HVA (15).

retention times were reduced but overlapping of major metabolites was also noticed.

The resolution of amines and acidic metabolites also depends upon the acidity of the eluent [2,16,17]. Lowering the pH of the eluent tends to suppress the degree of dissociation of the acidic metabolites such as DOPAC, HIAA and HVA. Consequently, acidic metabolites retained longer on the reversed-phase column. Slight adjustment of pH was

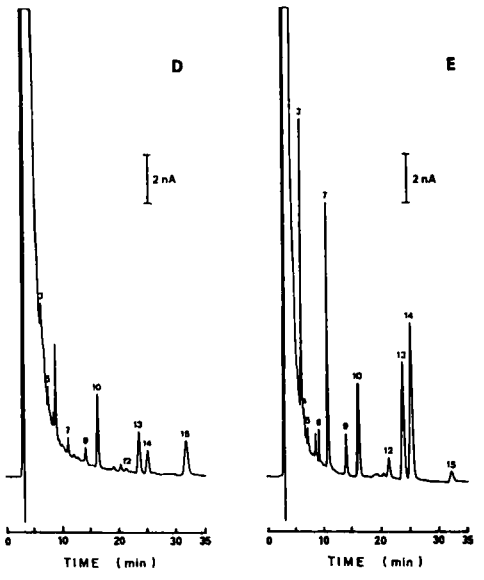

FIGURE 1 (continued)

associated with poor resolution and significant changes in the retention times of peaks. Under the present chromatographic conditions, it is critical that pH of the mobile phase be fine adjusted to 3.7 to optimize the separation and resolution of neurotransmitters. TEA in the mobile phase helped to prevent tailing of peaks and EDTA served to chelate heavy metal ions, reducing background currents.

The observation that NE, DA, HIAA and 5HT levels were high (Fig1E) in rat brain was not unique. Similar observation have been reported by others [7,15]. The reliability of our assay was also dependent on using ISOP as the internal standard. In preliminary studies, we have tested other internal standards such as DHBA and 5-HI, but they either overlapped with a major peak of interest or a component of

the tissue constituents. Ascorbic acid, an anti-oxidant, was added in our samples to prevent the break down of standards and neurotransmitters [18]. In this procedure, we used volatile formic acid for not being detrimental to the LC-EC system [15,19].

In conclusion, we have established a chromatographic assay for baseline to baseline separation of biogenic amines and their major metabolites simultaneously after a direct injection of PCA precipitated supernatant. Due to the simplicity, sensitivity and rapid acquisition of data in our assay condition, we believe this procedure can be applied for the analysis of CSF and brain, permitting more thorough studies of the relation and interaction between different monoamine neurotransmitter systems and their metabolites.

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REFERENCES

(1) Mefford, I.N., Application of high performance liquid chromatography with electrochemical detection to neurochemical analysis: Measurement of catecholamines, serotonin and metabolites in rat brain, J. Neurosci. Meth., 3, 207, 1981.

(2) Krstulovic, A.M., Investigations of catecholamine metabolism using high-performance chromatography : Analytical methodology and clinical applications, J. Chromatogr., 229, 1, 1982.

(3) Hjemdahl, P., Catecholamine measurements by high performance liquid chromatography, Am. J. Physiol., 247, E13,1984.

(4) Kissinger, P.T., Biomedical application of liquid chromatography - electrochemistry, J. Chromatogr., 488, 31, 1989.

(5) Magnusson, O., Nilsson, L.B., and Westerlund, D., Simultaneous determination of dopamine, DOPAC and homovanillic acid : Direct injection of supernatants from brain tissue homogenates in liquid chromatography - electrochemical detection system, J. Chromatogr., 221, 237, 1980.

(6) Kilts, C.D., Breese, G.R., and Mailman, R.B., Simultaneous quantification of dopamine, 5-hydroxytryptamine and four metabolically related compounds by means of reversed-phase high-performance liquid chromatography with electrochemical detection, J. Chromatogr., 225, 347, 1981.

(7) Kim, C., Campanelli, C., and Khanna, J.M., Determination of picogram levels of brain catecholamines and indoles by a simplified liquid chromatographic electrochemical detection method, J. Chromatogr., 282, 151, 1983.

(8) Lasley, S.M., Michaelson, I.A., Greenland, R.D., and McGinnis, P.M., Simultaneous measurement of tyrosine, tryptophan and related monoamines for determination of neurotransmitter turnover in discrete rat brain regions by liquid chromatography with electrochemical detection, J. Chromatogr., 305, 27, 1984.

(9) Saller, C.F., and Salama, A.I., Rapid automated analysis of biogenic amines and their metabolites using reversed-phase high performance liquid chromatography with electrochemical detection, J. Chromatogr., 309, 287, 1984.

(10) Kotake, C., Heffner, T., Vosmer, G., and Seiden, L., Determination of dopamine, norepinephrine, serotonin and their major metabolic products in rat brain by reverse-phase ion-pair high performance liquid chromatography with electrochemical detection, Pharmacol. Biochem. Behav., 22, 85, 1985.

(11) Elrod, L. Jr., and Mayer, G.L., Determination of monoamine metabolites in human cerebrospinal fluid by high performance chromatography with electrochemical detection, J. Liq. Chromatogr., 8, 1817, 1985.

(12) Wagner, J., Vitali, P., Palfreyman, M.G., Zraika, and M., Hout, S., Simultaneous determination of 3,4-dihydroxy-phenylalanine, 5-hydroxytryptophan, dopamine, 4-hydroxy-3-methoxy-phenylalanine, homovanillic acid, serotonin and 5-hydroxyindoleacetic acid in rat cerebrospinal fluid and brain by high-performance liquid chromatography with electrochemical detection, J. Neurochem., 38, 1241, 1982.

(13) Wanhoff, M., Simultaneous determination of norepinephrine, dopamine, 5-hydroxytryptamine and their main metabolites in rat brain using high performance chromatography and electrochemical detection : Enzymatic hydrolysis of metabolites prior to chromatography, J. Chromatogr., 307, 271, 1984.

(14) Hadfield, M.G., Crane, P., King, M.E., Nugent, E.A., Milio, C., and Narasimhachari, N., Determination of 13 catecholamines, indoleamines, metabolites and precursors in less than 20 minutes during a single HPLC run, J. Liq. Chromatogr., 8, 2689, 1985.

(15) Hong, C.S., Bush, B., Seegal, R.F., and Brosch, K.O., Determination of biogenic amines and their metabolites in brain, cerebrospinal fluid and plasma, Anal. Lett., 20, 435, 1987.

(16) Nagao, T., and Tanimura, T., Simultaneous determination of biogenic amines, their precursors and metabolites in a single brain of the cricket using high-performance liquid chromatography with amperometric detection, J. Chromatogr., 496, 39, 1989.

(17) Knox, J.H., and Hartwick, R.A. Mechanism of ion-pair liquid chromatography of amines, neutrals, zwitterions and acid using anionic hetaerons, J. Chromatogr., 204, 3, 1981.

(18) Verbiese-Genard, N., Hanocq, M., Alvoet, C., and Molle, L., Degradation study of catecholamines, indoleamines and some of their metabolites in different extraction media by chromatography and electrochemical detection, Anal. Biochem., 134, 170, 1983.

(19) Rossi, G., Deppieri, O., and Pessina, A.C., Effect of haemolysis on high performance liquid chromatography measurements of free plasma catecholamines, J. Chromatogr., 380, 117, 1986.