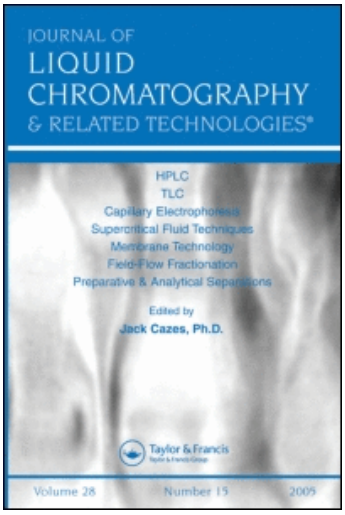

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Fast HPLC Analysis Of Catecholamines and Indoleamines in Avian Brain Tissue

G. F. Barbato[a]
[a] Department of Poultry Science and Inter-College Graduate Programs in Genetics and Physiology, The Pennsylvania State University, University Park, Pennsylvania

To cite this Article Barbato, G. F.(1990) 'A Fast HPLC Analysis Of Catecholamines and Indoleamines in Avian Brain Tissue', Journal of Liquid Chromatography & Related Technologies, 13: 13, 2553 — 2560
To link to this Article: DOI: 10.1080/01483919008049054
URL: http://dx.doi.org/10.1080/01483919008049054

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(13), 2553-2560 (1990)

A FAST HPLC ANALYSIS OF CATECHOLAMINES AND INDOLEAMINES IN AVIAN BRAIN TISSUE

G. F. BARBATO
Department of Poultry Science
and Inter-College Graduate Programs
in Genetics and Physiology
The Pennsylvania State University
University Park, Pennsylvania 16802

ABSTRACT

A fast HPLC method for the determination of monoamine levels in avian brain tissue, using a short 3 micron column and electrochemical detection, is presented. This system simultaneously analyzes 18 catecholamines, indoleamines, metabolites, and internal standards, without further sample preparation, in 20 minutes. Contrary to reports involving the mammalian species, incubation of the tissue homogenate with ascorbate oxidase and/or urate oxidase does not significantly alter the analysis under the reported conditions. The assay was used to evaluate differences in the neurochemical content of brain tissue from chickens divergently selected for 42 day exponential growth rate.

INTRODUCTION

A number of assays currently exist which simultaneously quantitate catecholamines and indoleamines in brain tissue. The majority of these assays have been developed specifically for use with the mammalian species (e.g., 1,

2, 3). Very few studies have been published relating HPLC techniques with the avian species.

Most research involving monoamines in chickens use radioenzymatic and fluorometric techniques (e.g., 4, 5, 6). In this study I am reporting refinements of methods used with the mammalian species applied to chicken brain tissue. This method is specifically being designed to investigate neurotransmitter metabolism in populations of chickens that have undergone divergent selection for growth rate.

MATERIALS AND METHODS

Instrumentation: The HPLC system (see Figure 1) consists of a Rainin Rabbit pump and pressure monitor, a Gilson 401/231 autosampler (with a 5μl injection loop), and a Gilson 141 electrochemical detector. The system was controlled and data collected by the Gilson 714 HPLC system controller on an IBM PS/2 Model 30. Mobile phases were filtered and degassed using the Kontes Ultra-Ware HPLC mobile phase degassing system. The system is plumbed so the effluent from the detector is recirculated to the mobile phase reservoir. The system is equilibrated overnight (at +800 mV; 500 nA/V), allowing the electrochemical cell to oxidize any remaining electrochemically active contaminants in the mobile phase. This has consistently provided a stable, low-noise baseline.

In order to provide the fastest assay with the greatest separative capacity, we used a short, three micron column (Rainin Dynamax 10 x 0.46 cm, C_{18}, reversed-phase) with a 3 cm guard column of the same packing.

Chemicals: All standards were purchased from the Sigma Chemical Co. (St. Louis, MO). Standards, abbreviations, respective elution times, and standard curves are presented in Table 1. HPLC-grade acetonitrile, diethylamine, solid NaOH, and citric acid were obtains from Baker Chemical Company. HPLC-grade sodium acetate and HPLC-grade glacial acetic acid were obtained from Fisher Scientific. Sodium octyl sulfate, an ion-pairing agent, was obtained from Aldrich Chemical Co. Ascorbate oxidase, urate oxidase, and $Na_2EDTA*2H_2O$ were obtained from Sigma Chemical Co.

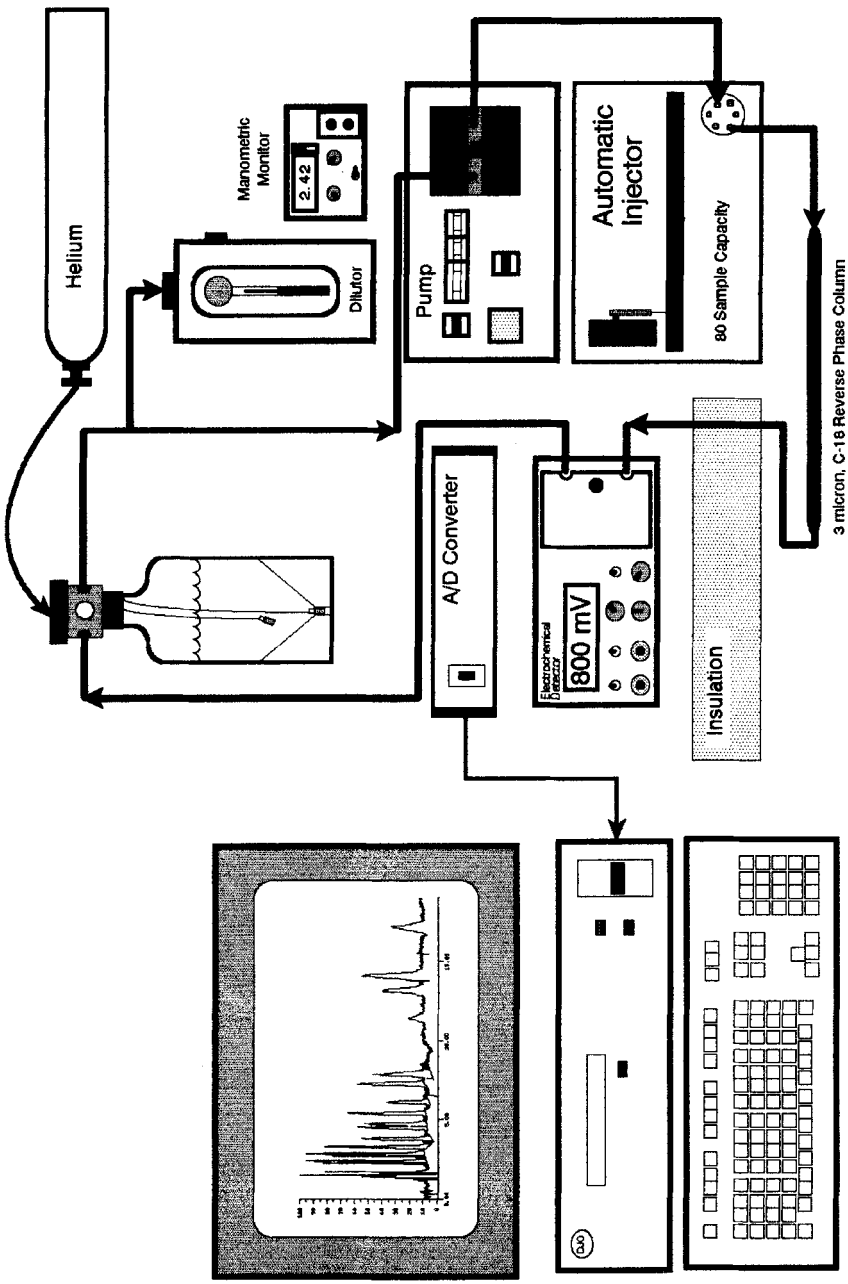

Figure 1. Isocratic HPLC system used to quantitate monoamines. (Inset is actual chromatogram of standards -- 200pg/std.)

TABLE 1

NEUROCHEMICAL STANDARDS, ABBREVIATIONS, RETENTION TIMES (RT) AND REGRESSION COEFFICIENTS

NEUROCHEMICAL	ABBREV.	RT	β	α	R^2
3,4-Dihydroxyphenylglycol	DOPEG	1.48	located in tissue front		
Vanillylmandelic acid	VMA	1.94	0.0099	25.3	0.988
3-Methoxy-4-hydroxyphenylglycol	MHPG	2.05	0.0069	-82.2	0.998
Norepinephrine	NE	2.49	0.0109	-111.5	0.997
Epinephrine	EPI	3.08	0.0065	-41.1	0.998
3,4-Dihydroxybenzylamine	DHBA[1]	3.56	0.0081	-3.4	0.986
3,4-Dihydroxyphenylacetic acid	DOPAC	3.73	0.0086	-116.8	0.934
3,4-Dihydroxyphenylalanine	DOPA	3.85	0.0083	-122.4	0.945
Normetanephrine	NM	4.24	0.0079	-155.4	0.940
Dopamine	DA	5.06	0.0048	18.1	0.999
5-Hydroxytryptophol	5-HTOL	5.58	0.0082	-54.3	0.956
Metanephrine	MET	6.02	0.0078	-148.3	0.980
3-Hydroxyindole-3-acetic acid	5-HIAA	6.74	0.0060	-148.6	0.981
3-Methoxy-4-hydroxyphenylacetic acid	HVA	9.39	0.0121	-2.7	0.999
5-Hydroxytryptophan	5-HTP	11.72	0.0047	6.6	0.997
3-Methoxytyramine	3-MT	13.12	0.0040	123.6	0.994
5-Hydroxytryptamine	5-HT	14.52	0.0032	-89.8	0.950
N-Methyl-5-hydroxytryptamine	N-MET[1]	18.45	0.0060	120.8	0.994

[1]Internal standards

Distilled water was deionized, passed over an organic cartridge (Corning) and steam distilled on glass. Only freshly distilled water (<24 hrs) was used to prepare the mobile phase and extraction buffers. All buffers were prepared just prior to analysis.

Chromatographic Conditions: The mobile phase is always prepared in four (4) liter batches in the following manner (modified from 7). One gram of solid NaOH is dissolved in 3750 ml freshly distilled H_2O. Upon dissolution, 74.5 mg Na_2EDTA is added, followed by 84 g citric acid, 3.4 ml diethylamine (DEA) and 209 mg of sodium octyl sulfate. The mobile phase is then filtered (45 mm, 0.45 micron Gelman Vericel filter; FP-450) and degassed directly into a five (5) liter HPLC mobile phase reservoir. The pH of the solution is 2.65. Two hundred and fifty (250) ml of acetonitrile are added directly to the

HPLC reservoir, which is constantly stirred and placed under a helium blanket. Flow rate was set at 1.2 ml/min and separations performed at ambient temperature. The detector was set at an oxidizing potential of 750 mV at 2 nA/V. Standard curves were developed for each neurochemical and metabolite using masses ranging from 10 - 1000 pg (see Table 1). In order to accurately quantify the neurochemicals in the brain, which vary widely in concentration, data was collected at both 10 and 100 mV full scale.

Tissue Preparation and Enzyme Treatment: Five 14-day old male chicks were killed via cervical dislocation and the brains were rapidly removed and frozen on dry ice. This tissue was used to determine if ascorbic acid or uric acid were interfering with the assay. The brains were kept in a -80C freezer prior to analysis. The tissue was weighed, extracted in 10 w/v of 25 mM acetate extraction buffer (pH = 5.0 with HPLC grade glacial acetic acid), and homogenized using a Tekmar Tissumizer at high speed for 30 seconds. A one (1) ml aliquot of the homogenate was removed and frozen for subsequent protein determination (8). The homogenate was then spun at high speed for one (1) minute in a refrigerated Savant High Speed centrifuge. Ten 100 μl aliquots (n = 10/treatment) of the pooled supernatant was then incubated with 5 μl of an ascorbate oxidase solution (1 mg/ml), 5 μl of a urate oxidase solution (1 mg/ml), or 5 μl of each enzyme solution (2,9). Analysis was completed, as above, catecholamine mass calculated and corrected for volume, and expressed per mg brain protein. The values for VMA, MHPG and NE were analyzed via two-way anova of SAS, to determine the most effective enzyme treatment(s).

Incubation of the brain homogenate with ascorbate oxidase significantly reduced the tissue front, but since the baseline of the chromatogram equilibrated *after* the tissue front and *prior* to the peaks of interest, no advantage was realized from the use of the enzyme. Urate oxidase produced no change in the quantification of the neurotransmitters, although resulted in a very large, broad peak approximately 15 minutes following a run. The use of enzyme treatment during this assay is neither warranted nor recommended. I should point out, however, that prior to using a short 3μ column, we used a 25 x 0.46 cm, 5μ, C_{18} reversed phase column. When using this column, it was necessary to pre-treat the samples with

TABLE 2

HPLC MEANS AND STANDARD ERRORS FOR BRAIN MONOAMINES (pg/mg brain protein) FROM 14 DAY OLD CHICKS DIVERGENTLY SELECTED FOR 42 DAY EXPONENTIAL GROWTH RATE[1]

LINE	VMA	MHPG	DOPA	NE	5HIAA	EPI
42L	466±79^b	110±20^b	534±195	4520±500^b	2170±240^b	442±88^b
42H	318±54^a	54±18^a	365±185	3060±470^a	1550±230^a	176±130^a

LINE	DOPAC	NM	DA	MET	HVA	5HT
42L	541±291	677±268	908±127	862±136^b	1440±360	1030±130
42H	575±285	519±292	626±107	367±136^a	1330±330	950±120

[1]Column sub-groups having different superscripts are significantly different ($P<0.05$).

ascorbate oxidase to accurately quantify NE. Use of the 3μ column also allowed us to separate VMA and MHPG from the tissue front.

Populations tested: The animals used in this experiment were obtained from populations of chickens which were divergently selected for 42 day exponential growth rate. This resulted in two groups of chickens having very fast and very slow growth (42H and 42L, respectively). At 14 days of age, 42H chicks were 30% larger than 42L chicks (Barbato, unpublished observations). Brain tissue was obtained from chicks of both sexes from these lines of at 14 days of age (n = 10 chicks/line/sex). The tissue was stored and extracted as described in the previous section.

RESULTS AND DISCUSSION

We were able to quantify 12 neurotransmitters and metabolites in the avian brain. At 14 days of age, there were no differences in the level of neurochemicals due to sex, so all values were pooled and presented in Table 2 by line only. Significant differences were observed for many of the neurochemicals. Notably, concentration of NE, EPI and their metabolites (MET, VMA, and MHPG) were significantly higher among 42L chicks than their 42H counterparts. Dopamine and HVA were not significantly different

between the populations. While 5HT was not significantly different between the populations, 5HIAA concentration was significantly higher among 42L chicks.

The current method is clearly sensitive enough to detect low levels of neurotransmitters in the brain and consistent enough to evaluate differences among populations of chickens. Further, the pattern of significance among the metabolites suggests that artificial selection for growth has altered neurotransmitter metabolism. In this regard, the proposed assay has the capability to estimate the activity of several enzymes involved in neurotransmitter metabolism using established incubation techniques (7).

In order to increase the separative capacity of the assay, we have recently changed the mobile phase as follows: pH = 2.75; 3% acetonitrile; and flow rate = 1.3 ml/min. This has allowed us to additionally separate tyramine, epinine (deoxyepinephrine), vanillic acid, and tyrosine, without any co-eluting peaks. At this point in time, DOPEG and DOMA (3,4-dihydroxymandelic acid) elute in the front peak.

REFERENCES

1. Shihemi, E., Segal, M., and Jacobowitz, D. M., Application of high performance liquid chromatography with electrochemical detection to the determination of catecholamines in microdissected regions of the rat brain, *J. Neurosci. Methods.*, 8, 275, 1983.

2. McKay, L., Bradberry, C., and Oke, A., Ascorbic acid oxidase speeds up analysis for catecholamines, indoleamines and their metabolites in brain tissue using high-performance liquid chromatography with electrochemical detection, *J. Chromatogr.*, 311, 167, 1984.

3. 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. Liquid Chromatogr.*, 8, 2689, 1985.

4. Juorio, A. V., and Vogt, M. Monoamines and their metabolites in the avian brain. *J. Physiol.*, 189, 489, 1967.

5. Ahtee, L., Sharman, D. F., and Vogt, M., Acid metabolites of monoamines in avian brain; effects of probenecid and reserpine, *Br. J. Pharmac.*, 38, 72, 1970.

6. Macnamee, M. C., and Sharp, P. J., The functional activity of hypothalamic dopamine in broody bantam hens. *J. Endocrinol.*, 121, 67, 1989.

7. Blank, C. L., Wong, P., Bulawa, M., and Lin, P. The determination of catecholamine and indoleamine enzymatic activities using liquid chromatography with electrochemical detection, in Usdin, E., Wiener, N., and Youdim, M. B. H., eds., **Function and regulation of monoamine enzymes: Basic and clinical aspects.**, MacMillan Publishers, Ltd., London, 1981.

8. Markwell, M. A. K., Haas, S. M., Tolbert, N. E., and Bieber, L. L., Protein determination in membrane and lipoprotein samples: Manual and automated procedures. *Methods in Enzymology*, 72, 296, 1981.

9. Hadfield, M. G., Milio, C., and Narasimhachari, N., Simultaneous HPLC analysis of catecholamines and indoleamines in mouse brain tissue following acetate extraction and treatment with ascorbate oxidase, *J. Liquid Chromatogr.*, 10, 2439, 1987.