Pharmacology Biochemistry & Behavior, Vol. 18, pp. 943–948, 1983. © Ankho International. Printed in the U.S.A.

A Method for Manual Determination of Glutamate, Glutamine, GABA and Aspartate from Brain Regions[1]

ALANE S. KIMES[2] AND M. KENT SHELLENBERGER

Department of Pharmacology and the Ralph L. Smith Center for Mental Retardation Research
University of Kansas College of Health Sciences, Kansas City, KS 66103

Received 16 October 1982

KIMES, A. S. AND M. K. SHELLENBERGER. *A method for manual determination of glutamate, glutamine, GABA and aspartate from brain regions.* PHARMACOL BIOCHEM BEHAV 18(6) 943–948, 1983.—An improved two column method for rapid manual determination of glutamate (Glu), glutamine (Gln), γ-aminobutyric acid (GABA) and aspartate (Asp) from brain regions is presented. The method uses a Dowex-1 column and various concentrations of acetic acid to separate Glu, Asp and the neutral amino acids. The neutral amino acids are subsequently placed on a Dowex-50 column from which potassium acetate buffers are used to separate the amido amino acids, GABA and the other neutral amino acids. The amido amino acids are hydrolysed in NaOH and the glutamine derived glutamate is separated from contaminants on another Dowex-1 column. Amino acids are manually quantitated using the fluorescence from the reaction with *o*-phthaldialdehyde. The method described in this paper requires no special equipment other than a fluorometer. The procedure was designed to determine levels of amino acid neurotransmitters and catecholamines from a single brain sample as small as 0.12 g, however, due to the sensitivity of the *o*-phthaldialdehyde reaction, levels of the four amino acids could be determined on a sample one tenth that size or smaller.

Quantitation of amino acid neurotransmitters Glutamate, glutamine, GABA and aspartate Brain regions
Column chromatography *o*-Phthaldialdehyde

THERE is a growing literature indicating that glutamate (Glu) and aspartate (Asp) are neurotransmitters in some pathways of mammalian brain, thereby joining γ-aminobutyric acid (GABA) in the more or less established category. These amino acids are metabolically linked via the tricarboxylic acid cycle and the GABA shunt. Glutamine (Gln) may be viewed as an indicator of changes in flux through compartments containing Glu and GABA [5]. As a result, it is of interest to be able to isolate and quantitate these four amino acids in one procedure. Most procedures currently used for quantification either concentrate on a single amino acid [11] or yield data on large numbers of amino acids [19]. Enzymatic procedures [9, 10, 16] possess sensitivity and technical difficulty beyond that required for many applications. Other procedures require extensive and expensive instrumentation [12,21]. A technically simple method which retains good sensitivity seemed desirable for application to physiologic, pharmacologic and toxicologic studies.

The method described in this paper is manual, requires no special equipment other than a fluorometer and is relatively quick. The number of samples which can be processed is limited only by the number of columns which can be operated simultaneously. The procedure uses two short columns, one anionic and one cationic, to separate the strongly acidic and neutral amino acids as first proposed by Hirs, Moore and Stein [14], and modified by Berl, Lajtha and Waelsch [5]. The present method has further modifications which speed the separations. This method also incorporates a manual version of the automated *o*-phthaldialdehyde fluorescent procedure of Benson and Hare [4], which was adapted from the original procedure of Roth [20].

METHOD

Preparation of Resins

Materials. Dowex-1 resin, 8% cross linked, 400 mesh, purchased in the chloride form. Dowex-50 resin, 8% cross linked, 400 mesh, purchased in the hydrogen form.

Solutions. Saturated NaCl, 2 N NaOH, 2 M Acetic Acid (HAc), 4 N HCl, 0.2 M Sodium acetate (NaAc) buffer at pH 4.4.

Dowex-1. To convert new resin to the acetate form, the resin was initially wetted with saturated NaCl solution and packed in a large column (in this case 36×360 mm), allowing enough room for expansion of the resin with washing. The resin was first converted to the hydroxyl form by dripping 4 liters of 2 N NaOH through the column at a rate of 40 ml/hr followed by washing with distilled water until the pH

[1]These studies were supported by U.S. Public Health Service Grant AM-27739 and Research Service Award HD-07066 (ASK).
[2]Present Address: Dept. Renal Medicine, Chesapeake Physicians Association, Baltimore City Hospitals, Baltimore, MD 21224.

dropped to between 7 and 8. The resin was then removed from the column and conversion to the acetate form started in a large flask by mixing with equal volumes of 2 M HAc until the resin changed from a dark yellow to a pale yellow and no longer bubbled. The resin was then repacked in a large column and 18 liters of 2 M HAc dripped through at the rate of 50 ml/hr to complete the conversion to the acetate form. This procedure was more efficient in removing Cl^- than the previously recommended procedure [7]. The converted resin was stored under 2 M HAc, until needed. To regenerate used resin, the above procedure was followed omitting the wetting step.

Dowex-50. New resin was wetted with 4 N HCl and washed with 4 N HCl until the supernatant fluid was clear, decanting the lighter debris with the supernatant fluid. The resin was then washed in distilled water, with decantation, until neutral pH was reached, after which it was washed in acetone, followed by another distilled water wash. The resin was converted to the Na form by washing with 2 N NaOH (3 vol) until alkaline pH was reached. Finally, it was washed with 0.2 M sodium acetate (NaAc) buffer (pH 4.4) until the pH was 4.4 and stored under this buffer until needed. Procedures for regenerating used resin are the same as above, starting with the 4 N HCl.

Preparation of Columns

Materials. Kontes Chromoflex disposable columns (K-420160-0000) (Kontes Glass, Vineland, NJ). Pasteur capillary pipettes, 9 inch (inside diameter 0.6 cm). Glass wool.

Columns for Dowex-1 were assembled from Kontes Chromoflex parts. This tubing was 8.5×0.8 cm and a funnel reservoir was used. The columns were plugged with glass wool and a slurry of Dowex-1 in acetic acid was poured into the column and allowed to settle. The height was then adjusted to 8.0 cm and the columns (8.0×0.8 cm) were washed with 20 ml 2 M HAc and 10 ml distilled water before use. Disposable 10 microliter pipettes were inserted into the column tips. The flow rate through the column was usually 12–14 drops/min although occasionally it was necessary to repour the column after adjusting the glass wool packing to attain this rate of flow.

Columns for Dowex-50 were assembled from 9 inch disposable Pasteur pipettes (0.6 cm, i.d.) and Kontes Chromoflex funnel reservoirs. The pipettes were plugged with glass wool and a slurry of Dowex-50 in 0.2 M NaAc buffer (pH 4.4) was poured into the pipette and allowed to settle. Column height was adjusted to 7.0×0.6 cm and the column washed with 20 ml of 0.2 M NaAc buffer (pH 4.4). Flow rate through this column was also required to be in the range of 12–14 drops/min and adjustment of the glass wool plug was occasionally necessary.

Tissue Preparation

Reagents and solutions. Acetone, solid CO_2, isopentane (2-methyl butane), 0.4 N perchloric acid (PCA).

Adult (4 months old) male and female Sprague-Dawley rats were bred in this laboratory from Charles River stock. The rats were killed by decapitation and the brains removed and frozen in less than 90 sec in isopentane cooled in an acetone-solid CO_2 bath [1]. The brains were stored at −70°C but were thawed to −16°C before being cut into 2–3 mm coronal sections using a multi-bladed knife. Individual regions were dissected from these sections on a glass stage over solid CO_2. The regional samples were homogenized in 3 ml 0.4 N PCA as described by Shellenberger and Gordon [22] for extraction of catecholamines. After 10 min in ice, the homogenates were centrifuged at 20,000–28,000 × g for 15 min with the pellet being rehomogenized in 2.5 ml 0.4 N PCA and centrifuged as above. The supernatant fluids were combined and the final volume adjusted to 6 ml. Of this extract, 0.5 ml was used for amino acid determination and the remainder for catecholamine and 5-hydroxytryptamine determination [22].

Elution of Amino Acids

Reagents and solutions. Acetic acid (HAc) solutions of varying molarity (Table 1), 0.2 M potassium acetate (KAc) buffers of varying pH (Table 1), 2.0 M NaOH, four standard solutions containing Glu, Gln, GABA and Asp: A, B, C and D, containing respectively 0.025, 0.05, 0.1, or 0.2 μmole/ml 0.4 N PCA of all four amino acids (these solutions were kept as frozen aliquots until the day of the column run), 0.5 ml of each tissue extract.

The 0.5 ml aliquot of the tissue PCA supernate was placed on the Dowex-1 column without neutralization. The amino acids were eluted with acetic acid by increasing the molarity (Table 1-A). Gln, GABA and other neutral amino acids were washed through the column in the first 3 ml (fraction B) and retained while the subsequent fraction was discarded. By increasing the HAc molarity to 0.5 M, Glu was eluted (fraction E) and a further increase to 1.0 M eluted aspartate (fraction G).

Fraction B, containing GABA and Gln, was transferred to the Dowex-50 column and the amino acids were separated by displacement with 0.2 M potassium acetate buffers of increasing pH (Table 1-B). The first 3 ml of eluate which contained serine (Ser), asparagine (Asn) and Gln (fraction 2) was retained. The other neutral acids were eluted in subsequent volumes of 0.2 M KAc, pH 4.4, with phenylalanine being the last to transit. If desired, the completeness of this step may be checked by spiking the samples with phenylalanine and spot checking the elution. When all phenylalanine had been removed, the pH was increased to 5.2 and GABA was eluted (fraction 5). Fraction 2 (containing Gln, Asn and Ser) was applied to a second Dowex-1 column for desalting. The Gln, Asn and Ser were again eluted by the addition of 3 ml of 0.1 M HAc (fraction B'). Fraction B' was evaporated overnight at 100°C, then hydrolyzed with 0.4 ml of 2 M NaOH for 1 hr at 100°C. The solution was neutralized with 2 M HAc and hydrolyzed amino acids were applied to a third Dowex-1 column. The Glu arising from the hydrolyzed Gln was eluted as before (Table 1-A). The eluted samples were capped and refrigerated until assayed and could be stored up to two days without loss of the amino acids. 0.5 ml aliquots of standard solution C and one of the other standard solutions were chromatographed and assayed with each set of tissue extracts.

Estimation of Amino Acids

Reagents and solutions. Borate buffer, 0.2 M, pH 9.7 containing 1.0 g/L polyoxyethylene-23-lauryl ether (Brij 35) which may be stored 2–3 days under refrigeration, and 1 mg/ml mercaptoethanol which is added to the borate buffer on the day of assay.

o-Phthaldialdehyde (OPT) (Sigma, St. Louis, MO) stock solution of 6 mg/ml in 95% ethanol which may be stored 1 week under refrigeration. This solution is diluted 1:10 in borate buffer just prior to use. Four standard solutions each for

TABLE 1

ELUTION SCHEME FOR ASP, GLU, GLN AND GABA FROM PCA EXTRACT OF NEURAL TISSUE

Step 1: Dowex-1 Column

Fraction	Solution Added	Compounds Eluted
A	0.5 ml PCA supernatant fluid	
B	3.0 ml, 0.1 M HAc	Gln, Asn, Ser, GABA and other NAA*
C	3.0 ml, 0.1 M HAc	
D	3.0 ml, 0.5 M HAc	
E	3.0 ml, 0.5 M HAc	Glu
F	2.0 ml, 1.0 M HAc	
G	3.0 ml, 1.0 M HAc	Asp

Step 2: Dowex-50 Column

Fraction	Solution Added	Compounds Eluted
1	3.0 ml of B (Step 1)	
2	3.0 ml 0.2 M KAc buffer, pH 4.4	Gln, Asn, Ser
3	9.0 ml 0.2 M KAc buffer, pH 4.4	Phe, etc.
4	2.0 ml 0.2 M KAc buffer, pH 5.2	
5	3.0 ml 0.2 M KAc buffer, pH 5.2	GABA

Step 3: Dowex-1 Column

Fraction	Solution Added	Compounds Eluted
A'	3.0 ml of 2 (Step 2)	
B'†	3.0 ml 0.1 M HAc	Gln, Asn, Ser

*Neutral amino acids.

†Evaporate overnight, hydrolyze with 0.5 ml 2 M NaOH for 1 hr at 100°C, neutralize with 2 M HAc, reapply to Dowex-1 as in step 1, assay fraction E''.

Glu, Asp, and GABA containing 0.025, 0.05, 0.067 and 0.1 μmole/ml of the appropriate elution solution.

Procedure. Column eluates containing one amino acid (e.g., Glu) and buffer were warmed to room temperature prior to the *o*-phthaldialdehyde reaction. 100 μl of each eluate or standard solution was added to a 5.0 ml test tube containing 2.0 ml of borate buffer. This was followed by 0.5 ml of the OPT reagent. The resulting solution was vortexed to facilitate the reaction. Because peak fluorescence developed in 5 min with Glu and Asp and remained stable for approximately 20 min, several samples were developed at once. However, since fluorescence with GABA peaked within two minutes then declined steadily, these samples were developed and read singly or in pairs. Standard curves (Fig. 1) were constructed for every group of samples from the fluorescence versus concentration of amino acids dissolved in the respective elution solutions. Blanks were developed from eluates of columns which had not received tissue samples. Fluorescence was read in an Amino-Bowman Spectrophotofluorometer, 330 nm exitation, 465 nm emission. Amino acid concentrations in the samples were calculated from the standard curves and corrected for recoveries of standard solutions which were chromatographed and assayed with each set of tissue extracts.

RESULTS AND DISCUSSION

The present method differs from earlier two column methods in several ways [5,14]. First, since a much smaller volume of tissue extract (0.5 ml) was used, neutralization was not needed. Secondly, columns with smaller resin bed volumes were used. This permits the use of smaller elution volumes and reduces waiting for eluant passage which is the time-consuming aspect of manual column procedures. Furthermore, elution from the Dowex-1 column was initiated with HAc of higher molarity since the present interest was in Glu and Asp which adhere strongly to the column. This allowed the preceding amino acids to be stripped from the column using smaller volumes and thereby speeding the isolation of Glu and Asp.

Similarly, changes in the eluting procedure accelerated the isolation of GABA from the Dowex-50 column. First the elution process was initiated with buffers of higher pH to strip the unwanted neutral amino acids from the column. Secondly, K^+ buffers which have greater displacement capacities than Na^+ buffers were used [9].

As with any column procedure, preparation of the resins and columns continue to be time consuming. However, as described in the Method section, the time spent washing the resins can be minimized by preparing large volumes and using gravity flow washes. After resin preparation, approximately one day is required to make Dowex-1 and Dowex-50 columns for 24 determinations. The resins are removed from the columns after each run but may be regenerated 3–5 times. Once the columns are made, 20 tissue samples can be analyzed from homogenization through quantitation in 3 days if only Glu, Asp and GABA are determined. One extra

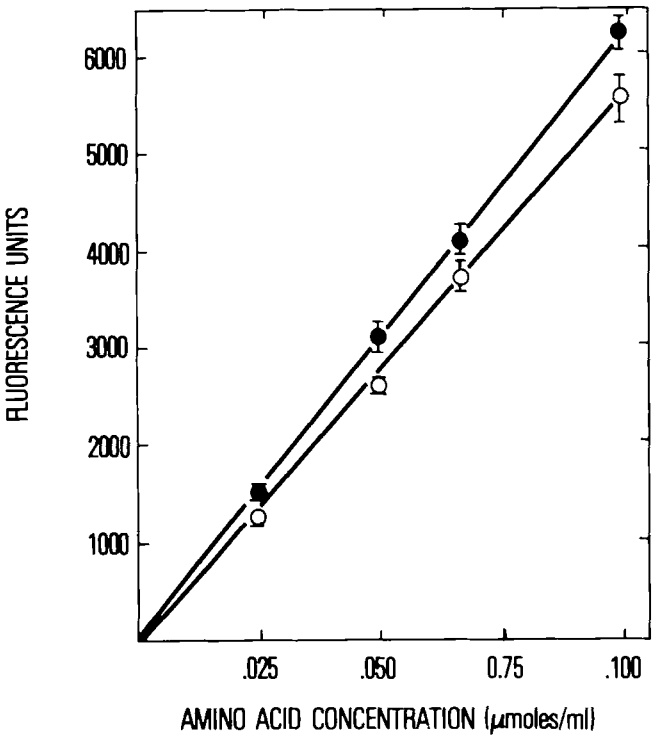

FIG. 1. Standard curves for the three amino acids. Fluorescence was read on an Aminco-Bowman Spectrophotofluorometer at the lowest photomultiplier gain. ●-Glu, r=0.9999, ○-GABA and Asp, r=0.9996. Data are given as means±S.E.M. N=27 to 33 determinations per point.

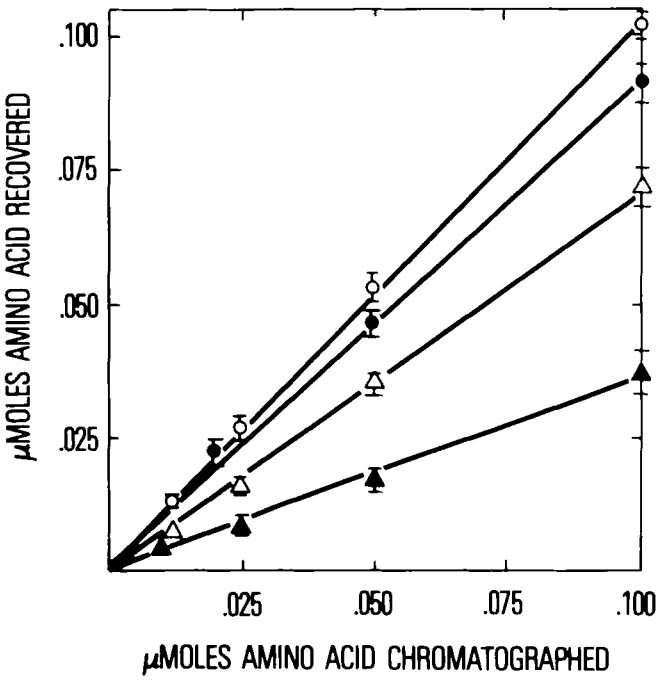

FIG. 2. Recoveries of standard amino acids using the two column method described in this paper. Linearity of recovery was evaluated by linear regression calculation. o-Glutamate, recovery 100%, r=0.9999; ●-Aspartate, recovery 95%, r=0.9995; △-GABA, recovery 70%, r=0.9990; ▲-Glutamine, recovery 35%, r=0.9980. All recoveries were significantly linear at $p<0.001$. Data are given as means± S.E.M. N=10 to 34 determinations per point.

day is required for Gln because of the hydrolysis and extra column steps.

The standard curves in Fig. 1 demonstrate the sensitivity of the OPT reaction. Fluorescence yields on amino acid standard solutions are quite high and linear from 0.25 to 0.100 μmoles/ml. The potential sensitivity for this manual procedure seems to be far greater than required in the present application. Because of the relatively high concentration of these amino acids in brain, levels in an eluant volume representing 0.7 mg of striatal tissue which is the smallest sample in our studies were readily quantitated. In Fig. 2 it can be seen that recoveries for each amino acid were also linear and reproducible across the concentrations studied. The recovery of GABA was somewhat reduced, possibly because of the column transfer step, but primarly because the elution peak tended to broaden and only the central 3 ml were used to ensure purity. Gln recoveries were reduced for two reasons. First, there was some loss due to the multiple column transfers and because the Dowex-50 column catalyzes deamidation to glutamate [23], most of which would elute after the Gln sample on the second Dowex-1 column. Further loss may have occurred during the evaporation step. In aqueous solutions at 100°C, Gln may form the cyclic compound: 5-oxo-2-pyrrolidine carboxylic acid [14] which in the presence of base may lose the carboxyl group. Nonetheless, because of the sensitivity of the OPT reaction and the quantity of Gln present in brain, the 35% recovery was adequate. A higher recovery may be possible with acid hydrolysis but the presence of Cl^- would present additional problems in the assay and the NH_4^+ could add to the background fluorescence. Lyophilizing instead of evaporating may also increase recoveries of Gln derived Glu.

The detection method used in this procedure is similar to the method originally published by Roth [20]. The major differences in the present procedure are the molarity and pH of the borate buffer, and the sequence of addition of reagents. The molarity (0.2 M), the addition of mercaptoethanol, the pH (9.7), and the addition of Brij were modifications made by Benson and Hare [4]. By adding the OPT separately after the amino acid and buffer had equilibrated, background fluorescence was reduced.

The substituted isoindole fluorescent adduct of OPT, mercaptoethanol and amino acid has been discussed by Chen *et al.* [6] and Cronin *et al.* [8]. The thermal stability of this product is related to the substitution on the carbon adjacent to the primary amine. Greater substitution leads to lower reactivity but greater stability once the complex is formed [8]. The different stabilities of the adducts formed with GABA versus Glu or Asp over time in this report may be the result of the substitution on the carbon adjacent to the amino group. This is the first report using OPT to assay all three amino acids in a non-automated procedure and therefore the first time the stability of the adduct was important to the assay procedure.

An example of data obtained from this procedure is shown in Table 2. These data are from 4-month-old male and female rats bred and reared in the Ralph L. Smith Center animal quarters. These data are consistent with and often identical to other values in the literature which were generated by a number of methods [2, 3, 13, 15, 18, 24] (Table 3).

A major advantage to the procedure described in this

TABLE 2

AMINO ACID LEVELS IN CONTROL RAT BRAINS

	N	Sample Wt.	Glutamate	Aspartate	GABA	Glutamine
			Cortex			
Male	8	180 ± 40*	10.82 ± 0.40†	4.32 ± 0.17	1.90 ± 0.07	4.52 ± 0.43
Female	4	165 ± 42	10.79 ± 1.19	3.91 ± 0.24	1.80 ± 0.10	5.35 ± 0.26
			Striatum			
Male	8	129 ± 23	8.92 ± 0.36	3.90 ± 0.13	2.65 ± 0.25	4.28 ± 0.76
Female	4	131 ± 22	8.14 ± 0.40	2.72 ± 0.02‡	2.11 ± 0.20	3.96 ± 0.58
			Midbrain			
Male	8	240 ± 46	6.09 ± 0.22	3.17 ± 0.15	3.89 ± 0.17	3.78 ± 0.38
Female	4	200 ± 14	6.02 ± 0.23	3.07 ± 0.15	3.74 ± 0.14	4.22 ± 0.30
			Pons-Medulla			
Male	8	222 ± 42	5.26 ± 0.17	3.67 ± 0.32	1.62 ± 0.11	4.12 ± 0.39
Female	4	245 ± 30	5.08 ± 0.10	3.00 ± 0.28	1.46 ± 0.09	3.59 ± 0.47

*Milligrams wet tissue ± standard deviation.
†μmoles/g wet tissue ± standard error.
‡n=3.

TABLE 3

PUBLISHED VALUES FOR AMINO ACID LEVELS† IN RAT BRAIN

Reference	Glutamate	Aspartate	GABA	Glutamine
		Cortex		
[2]			1.25 ± 0.04	
[3]	9.95 ± 0.42			
[15]	8.35 ± 0.97		1.67 ± 0.07	
[18]	12.87 ± 0.21	3.33 ± 0.12	1.87 ± 0.08	4.34 ± 0.19
[24]	10.64 ± 0.65	2.54 ± 0.29	1.85 ± 0.20	5.53 ± 0.33
		Striatum		
[2]			2.21 ± 0.08	
[3]	9.65 ± 0.58			
[15]	10.10 ± 0.45		2.71 ± 0.07	
[18]	11.08 ± 0.19	2.08 ± 0.07	2.47 ± 0.12	4.85 ± 0.23
		Midbrain		
[2]			3.14 ± 0.12	
[3]	8.18 ± 0.60			
[13]	5.84 ± 0.39	2.39 ± 0.00	3.21 ± 0.31	
[15]	9.74 ± 0.35		2.36 ± 0.10	
[24]	8.84 ± 0.62	2.94 ± 0.30	3.57 ± 0.48	5.41 ± 0.36
		Pons-Medulla		
[2]			1.54 ± 0.06	
[3]	6.02 ± 0.13			
[13]	6.01 ± 0.12	2.13 ± 0.16	1.35 ± 0.07	
[15]	6.61 ± 0.35		1.46 ± 0.07	
[18]	*7.25 ± 0.18	2.85 ± 0.10	2.07 ± 0.09	3.42 ± 0.11
[24]	5.27 ± 0.31	4.36 ± 0.34	1.44 ± 0.13	3.50 ± 0.19

*Brain Stem.
†μmoles/g wet tissue.

paper is its sensitivity. The procedure was designed for use with the catecholamine assay of Shellenberger and Gordon [22], utilizing only a small portion (0.5 ml) of the tissue extract. Used in this way, values for norepinephrine, dopamine, 5-hydroxytryptamine, Asp, Glu, GABA and Gln may be obtained from a single brain sample. The smallest tissue weight reported in this study is 129 mg (Table 2) of which one sixth of that weight is used for the amino acid determination. Therefore, the reported procedure could be used to determine amino acid concentrations in much smaller tissue samples if the extraction volume were reduced to 0.5–1.0 ml and used entirely for amino acid determination.

In this paper, a simple, sensitive, inexpensive two column procedure for isolating and quantitating amino acid neurotransmitters from several brain extracts simultaneously has been reported.

REFERENCES

1. Alderman, J. L. and M. K. Shellenberger. γ-Aminobutyric acid in the rat brain: Re-evaluation of sampling procedures and postmortem increase. *J Neurochem* **22**: 937–940, 1974.
2. Balcom, G. J., R. H. Lenox and J. L. Meyerhoff. Regional γ-aminobutyric acid levels in rat brain determined after microwave fixation. *J Neurochem* **24**: 609–613, 1975.
3. Balcom, G. J., R. H. Lenox and J. L. Meyerhoff. Regional glutamate levels in rat brain determined after microwave fixation. *J Neurochem* **26**: 423–425, 1976.
4. Benson, J. R. and P. E. Hare. *o*-Phthaladehyde: Fluorogenic detection of primary amines in the picomole range, comparison with fluorescamine and ninhydrin. *Proc Natl Acad Sci USA* **72**: 619–622, 1975.
5. Berl, S., A. Lajtha and H. Waelsch. Amino acid and protein metabolism. VI Cerebral compartments of glutamic acid metabolism. *J Neurochem* **7**: 186–197, 1961.
6. Chen, R. F., C. Scott and E. Trepman. Fluorescence properties of *o*-phthaldaldehyde derivatives of amino acids. *Biochem Biophys Acta* **576**: 440–455, 1979.
7. Cheng, S.-C. and H. Waelsch. Some quantitative aspects of the fixation of carbon dioxide by the lobster nerve. *Biochem Zeit* **338**: 643–653, 1963.
8. Cronin, G. R., S. Pizzaretti and W. E. Gandy. Amino acid analysis with *o*-phthalaldehyde detection: Effects of reaction temperature and thiol on fluorescence yields. *Anal Biochem* **93**: 174–179, 1979.
9. *Dowex: Ion Exchange*. The Dow Chemical Company, Midland, MI, 1959.
10. Graham, L. T., Jr. and M. H. Aprison. Fluorometric determination of aspartate, glutamate and γ-aminobutyrate in nerve tissue using enzymic methods. *Anal Biochem* **15**: 487–497, 1966.
11. Hare, T. A. and N. V. B. Manyam. Rapid and sensitive ion-exchange fluorometric measurement of γ-aminobutyric acid in physiological fluids. *Anal Biochem* **101**: 349–355, 1980.
12. Hassler, R., P. Haug, C. Nitsch, J. S. Kim and K. Paik. Effect of motor and premotor cortex ablation on concentrations of amino acids, monoamines, and acetylcholine and on the ultrastructure in rat striatum. A confirmation of glutamate as the specific corticostriatal transmitter. *J Neurochem* **38**: 1087–1098, 1982.
13. Himwich, W. A. and J. M. Davis. Brain amino acids as affected by acute and chronic administration of chlorpromazine. *Biol Psychiat* **5**: 89–98, 1972.
14. Hirs, C. H. W., S. Moore and W. H. Stein. The chromatography of amino acids on ion exchange resins. Use of volatile acids for elution. *J Am Chem Soc* **76**: 6063–6065, 1954.
15. Lenox, R. H., H. L. Wray, G. J. Kant, T. D. Hawkins and J. L. Meyerhoff. Changes in brain levels of cyclic nucleotides and gamma-aminobutyric acid in barbiturate dependence and withdrawal. *Eur J Pharmacol* **85**: 159–169, 1979.
16. Lowry, O. H. and J. V. Passonneau. *A Flexible System of Enzymatic Analysis*. New York: Academic Press, 1972.
17. Meister, A. *Biochemistry of the Amino Acids*. New York: Academic Press 1957, p. 7.
18. Nicklas, W. J. and S. Berl. *In vivo* studies on the effect of 6-hydroxydopamine on amino acid metabolism in brain. *Brain Res* **61**: 343–356, 1973.
19. Perry, T. L., D. Stedman and S. Hansen. A versatile lithium buffer elution system for single column automatic amino acid chromatography. *J Chromatogr* **38**: 460–466, 1968.
20. Roth, M. Fluorescence reaction for amino acids. *Anal Chem* **43**: 880–882, 1971.
21. Schmid, R., J. S. Hong, J. Meed and E. Costa. The effect of kainic acid on the hippocampal content of putative transmitter amino acids. *Brain Res* **200**: 355–362, 1980.
22. Shellenberger, M. K. and J. H. Gordon. A rapid simplified procedure for simultaneous assay of norepinephrine, dopamine and 5-hydroxytryptamine from discrete brain areas. *Anal Biochem* **39**: 356–372, 1971.
23. Stein, W. H. and S. Moore. The free amino acids of human blood plasma. *J Biol Chem* **211**: 915–926, 1954.
24. Zanchin, G., P. Rigotti, N. Dussini, P. Vassanelli and L. Battistin. Cerebral amino acid levels and uptake in rats after protocaval anastomosis: II Regional studies *in vivo*. *J Neurosci Res* **4**: 301–310, 1979.