Journal of Chromatography, 375 (1986) 37—47
Biomedical Applications
Elsevier Science Publishers B V , Amsterdam — Printed in The Netherlands

CHROMBIO 2891

QUANTIFICATION OF TRYPTAMINE IN BRAIN USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

ALICE A LARSON* and NELSON L DALO*

Department of Veterinary Biology, 295 Animal Science/Veterinary Medicine Building, University of Minnesota, St Paul, MN 55108 (U S A)

(First received June 18th, 1985, revised manuscript received September 27th, 1985)

SUMMARY

The concentration of endogenously formed tryptamine in central nervous system tissue was determined after extraction into ethyl acetate, purification on a weak cation-exchange resin and analysis using high-performance liquid chromatography with fluorometric detection Final chromatographic separation of this indoleamine was achieved using a μBondapak C_{18} reversed-phase column under isocratic conditions Using this method, the concentration of tryptamine in the whole brain of normal rats was found to be 0 60 ± 0 06 ng/g of tissue, while pretreatment with *dl-p*-chlorophenylalanine, tranylcypromine and *l*-tryptophan increased the concentration to 96 7 ± 21 9 ng/g

INTRODUCTION

Tryptamine (TA), an endogenous trace amine, is distributed non-homogeneously throughout the brain [1], has an apparent subcellular compartmentalization [2] and influences cortical neuronal firing rates [3] Reports of specific binding sites for TA [4], distinct from those of serotonin (5-HT) and other putative neurotransmitters [5], have recently strengthened the hypothesis that endogenously formed TA may play a role in neuroregulatory mechanisms in the central nervous system (CNS)

Methods for precise quantitation of the concentration and rate of turnover of TA are necessary for examination of the role of TA in the CNS Early techniques developed for the measurement of TA in the CNS either have inadequate

*Present address Escuela de Veterinaria, Universidad Centro Occidental, Barquisimeto, Venezuela

0378-4347/86/$03 50

sensitivity for the determination of tissue concentrations [6—8] or yield inflated estimations of this amine [9—12], perhaps due to contamination with other substances While more recent and accurate gas chromatographic—mass spectrometric (GC—MS) analyses of TA yield values between 0.05 to 0.5 ng/g, depending on the species tested [1, 13—16], the cost of determinations using this instrumentation typically prohibits widespread or routine use

The widespread use of column liquid chromatography and the unique characteristic of indoleamines to fluoresce at excitation maxima of 218—228 nm and 278—288 nm, with emission maxima at approximately 368 nm, has allowed the development of a sensitive and inexpensive method for the quantification of this indoleamine

EXPERIMENTAL

Reagents

HPLC-grade ethyl acetate, methanol and tribasic sodium phosphate A C S were purchased from Fisher Scientific (Fair Lawn, NJ, U S A) Dibasic potassium phosphate A.C S was obtained from Columbus Chemical Industries (Columbus, WI, U S A), monobasic sodium phosphate A R from Mallinckrodt (St. Louis, MO, U S A), sodium acetate A C.R and sodium metabisulfite from Matteson Coleman & Bell (Norwood, OH, U S A), formic acid A C.R from Baker (Phillipsburg, NJ, U S A) and trifluoroacetic anhydride (TFAA) from Pierce (Rockford, IL, U.S.A). The indoleamine standards (see also Table I), *dl-p*-chlorophenylalanine (PCPA) and tranylcypromine hydrochloride were purchased from Sigma (St Louis, MO, U S A.) The tryptoline derivatives were a kind gift from Dr Kym F Faul, Stanford University Medical Center (Stanford, CA, U S.A.)

Stock solutions of 1 mg/ml were prepared for each indole using 0.01 *M* hydrochloric acid or 0 01 *M* hydrochloric acid containing 5% methanol, as indicated for each compound in Table I, and kept at —4° C. A working solution of 1 μg/ml or 100 ng/ml of each compound was prepared just prior to the assay N-Acetyltryptamine (NATA) and N-acetyl-5-methyltryptamine were synthesized as described below by acetylation of TA and 5-methyltryptamine, respectively, according to the method of Warsh et al [16]

Preparation of Bio-Rex 70 precolumns

Bio-Rex 70 (200—400 mesh, Na^+), a weak cation-exchange resin (Bio-Rad Labs , Richmond, CA, U S A) was used The procedure used to isolate TA is a modification of the procedure previously described for the isolation of catecholamines [17] 5-HT [18—21] and TA [7, 16] The resin (200 g) was stirred for 30 min in 1 l of 3 *M* sodium hydroxide, allowed to settle and decanted. The resin was then washed three times with 1 l each of distilled water. The resin was converted to the acid form after stirring 30 min in 1 l of 3 *M* hydrochloric acid and then washed three times with 1 l per wash of distilled water The entire process was repeated once. After the last wash with water, the resin was placed in 0 2 *M* sodium potassium phosphate buffer and the pH adjusted to 6 1 by the addition of 0 2 *M* monobasic sodium phosphate The resin was stored in the refrigerator until use.

Polypropylene disoposable Econo-Columns (Bio-Rad Labs.) were filled with the resin which was then allowed to settle for 15 min. The volume was adjusted to between 0 7 and 0.8 ml and then the columns were washed with 10 ml of 0 2 *M* sodium potassium phosphate buffer (pH 6 1) and clamped off using a two-way Teflon stopcock until the samples were loaded. Nylon stopcocks were not used as they were found to react with strong acids and release fluorescent material that enhanced the background of the fluorometric determination.

Tissue extraction

Male Sprague—Dawley albino rats weighing between 250 and 300 g were killed by decapitation. The whole brain was removed and immediately frozen in liquid nitrogen. Brains were weighed and placed in 0.75 *M* tribasic phosphate buffer (pH 12 5), containing 10 g/l sodium metabisulfite, in a ratio of 2 ml of buffer per g of brain The tissue was homogenized on ice using an homogenizer for small samples (Biospec Products, Bartlesville, OK, U.S.A.), and then extracted using 2 ml of ethyl acetate per 1 ml homogenate. Tubes were shaken manually for 15 min, then centrifuged for 5 min at 3000 rpm (approx 1000 *g*) in a Beckman Model J-21B centrifuge (Beckman Instruments, Palo Alto, CA, U S.A) The organic layer was removed and the extraction repeated once. The ethyl acetate was pooled and evaporated using a Savant Speed Vac evaporator (Savant Instruments, Hickville, NY, U.S A) The residue was initially resuspended in 0 2 ml of 0 2 *M* phosphoric acid and then mixed with 9 8 ml of 0 2 *M* sodium potassium phosphate buffer (pH 6 1)

Precolumn clean-up

The solution obtained from the extraction of TA was filtered through a small amount of glass wool to retain gross residual particles and then placed directly onto Bio-Rex 70 columns, washed with 10 ml of 0 02 *M* sodium potassium phosphate buffer (pH 6 1) and then with 10 ml of HPLC-grade distilled water TA was eluted from the columns with 3 ml of 2 *M* formic acid by allowing five to six drops of the previous wash to pass through the column before clamping off for 30-min exposure to the acid. The columns were also clamped off for 5 min between the collection of each 1 ml of acid Two additional 0.5-ml volumes of 2 *M* formic acid were then applied to each column to wash the column The total 4 ml of eluent were collected in small glass tubes To decrease the elution time, the top of the resin could be gently stirred and the columns centrifuged at 500 rpm (approx 200 *g*) for 2—3 min with no effect on TA recovery. The eluent was dried in the Speed Vac evaporator and the residue resuspended in 100 μl of mobile phase for injection onto the reversed-phase column.

Apparatus

An isocratic high-performance liquid chromatographic (HPLC) system was used consisting of an Altex Model 110A pump (Altex, Berkeley, CA, U.S A.), a Rheodyne Model 7120 injector (Rheodyne, Berkeley, CA, U.S A.) and a Kratos Model GM 970 variable-wavelength fluorometer (Kratos Analytical Instruments, Ramsey, NJ, U.S A) as indicated The Kratos fluorometer was set at an excitation wavelength of 220 nm, a photomultplier tube appropriate for

a 300 nm emission cutoff, and a 350 nm bandpass, 25 nm width monochromatic filter

Separation of indoles was achieved using a 300 × 3 9 mm I D Waters μBondapak C_{18} column (Waters Assoc , Milford, MA, U S A) packed with 10-μm particles The mobile phase was a 0 01 *M* sodium acetate buffer (pH 4 6) containing 20% HPLC-grade methanol This buffer was prepared fresh each week and kept refrigerated until use Each day the mobile phase was filtered through a 0 3-μm Millipore filter (Millipore, Bedford, MA, U.S A.) and degassed. The flow-rate of the mobile phase was 2 5 ml/min Equilibration of the reversed-phase column was usually reached in 20—25 min

Derivatization

Acetylation of TA was accomplished where indicated by the method of Warsh et al [16] which is, in short, addition of two drops of 8 *M* sodium hydroxide to either 10 mg of the indoleamine or the collected, dried peak and then immediate extraction into 2 ml of anhydrous diethyl ether containing 2% acetic anhydride Samples are shaken for 15 min and the organic layer is transferred to a second tube. A second extraction is done with 2 ml of anhydrous diethyl ether only In our hands, the organic solvent was pooled and dried in a Speed Vac evaporator The residue was resuspended in 10 ml of 0 01 *M* hydrochloric acid containing 10% methanol and kept in the freezer at —4°C

Derivatization with TFAA is accomplished by the addition of 2 ml of TFAA—ethyl acetate (5 1, v/v) to each acetylated substance and allowed to react for 1 h at room temperature The TFAA—ethyl acetate was evaporated in a Speed Vac evaporator and resuspended in 10 μl ethyl acetate for GC–MS analysis GC was performed using a Series 530 methyl silicon column, 10 m × 0.53 mm I D., 2 65 μm film thickness (Hewlett-Packard, Avondale, PA, U.S A) The temperature of the injection port was set at 250°C and the flow-rate at 25 ml/min The temperature of the ion source was set at 225°C and the ionization energy at 50 eV. The β-carboline derivative was searched for by selected-ion monitoring (SIM) at *m/z* 280, 183, 154 and 115

RESULTS AND DISCUSSION

Sensitivity

The high natural fluorescence of indoleamines allowed us to exploit this characteristic of TA in the development of this assay The excitation wavelength of 220 nm excites indole compounds ten times more than the 280—285 nm range, which has been more commonly used in the past for measurement of TA [22, 23] Using the Kratos Spectrofluor monitor, supplied with a 300-nm photomultiplier tube (PMT) window and no emission filter, we were able to generate a linear standard curve with detection of 200 pg of TA at 220 nm using the column and mobile phase described in this assay. Use of a 350 nm bandpass monochromatic filter (25 nm width) decreased the second-order radiation that reached the PMT with a subsequent decrease in the background. This resulted in an extremely high sensitivity for TA, allowing us to detect amounts as low as 20 fg when injected directly into the detector and 20 pg when injected through the reversed-phase column using the conditions described in this report

Separation by organic solvent

A 0 01 *M* sodium acetate buffer (pH 4.6) containing 20% methanol was found to be a good mobile phase to achieve adequate separation of most indoleamines Methanol concentrations less than 20% achieved even better separation but decreased the peak height unacceptably The only compound of the 24 different indoles tested which overlapped with TA was N-acetyl-5-hydroxytryptamine (NA-5-HT), a melatonin precursor (Table I and Fig 1A). The overlap between NA-5-HT and TA was only partial but it became more pronounced as the column aged Using these chromatographic conditions, catecholamines, including epinephrine, norepinephrine, dopamine and 1-β-3,4-dihydroxyphenylalanine, were found to elute in or near the peak front, overlapping with 5-HT and 5-hydroxytryptophan but not with TA The fluorescence of catecholamines was extremely low when the excitation wavelength was set at either 285 or 220 nm and did not produce detectable fluorescence in amounts less than 400 ng Compounds such as tryptoline and methyltryptoline can be measured simultaneously with TA using 20% methanol, however, compounds such as tryptophol or melatonin, which have very long retention times, require methanol concentrations between 35 and 40% to achieve adequate sensitivity for detection of these endogenously formed compounds

TABLE I

RETENTION TIMES OF VARIOUS INDOLEAMINES

No	Compound	Retention time (min)	*n*
1	5-Hydroxytryptophan	1 90 ± 0 06	4
2	5-Hydroxytryptoline	2 07 ± 0 07	6
3	5-Hydroxytryptamine	2 15 ± 0 07	5
4	Indoleacetic acid aldehyde	2 36 ± 0 20	3
5	5-Hydroxymethyltryptoline	2 57 ± 0 08	8
6	*l*-Tryptophan	3 02 ± 0 23	11
7	5-Hydroxyindoleacetic acid	3 19 ± 0 12	4
8	5-Hydroxytryptophol	4 14 ± 0 38	10
9	*dl*-Methoxytryptophan	4 39 ± 0 17	5
10	Tryptamine	5 51 ± 0 13	14
11	N-Acetyl-5-hydroxytryptamine	5 88 ± 0 21	9
12	N-Methyltryptamine*	6 20 ± 0 24	7
13	5-Methoxytryptamine	6 75 ± 0 33	6
14	Tryptoline	8 70 ± 0 39	8
15	5-Methoxy-N,N-dimethyltryptamine*	9 10 ± 0 63	5
16	Indoleacetic acid*	10 57 ± 0 54	8
17	Methyltryptoline	11 32 ± 0 21	3
18	5-Methoxyindoleacetic acid	12 45 ± 0 21	3
19	5-Methyltryptamine*	12 85 ± 0 17	3
20	Tryptophol*	18 36 ± 0 97	6
21	5-Methoxytryptophol*	19 50 ± 0 61	10
22	N-Acetyltryptamine	25 78 ± 0 80	7
23	Melatonin*	27 70 ± 0 24	4
24	N-Acetyl-5-methyltryptamine	59 94	1

*Dilution of standard in 0 01 *M* hydrochloric acid containing 5% methanol All other compounds used as standards were dissolved in the acid solution containing no methanol

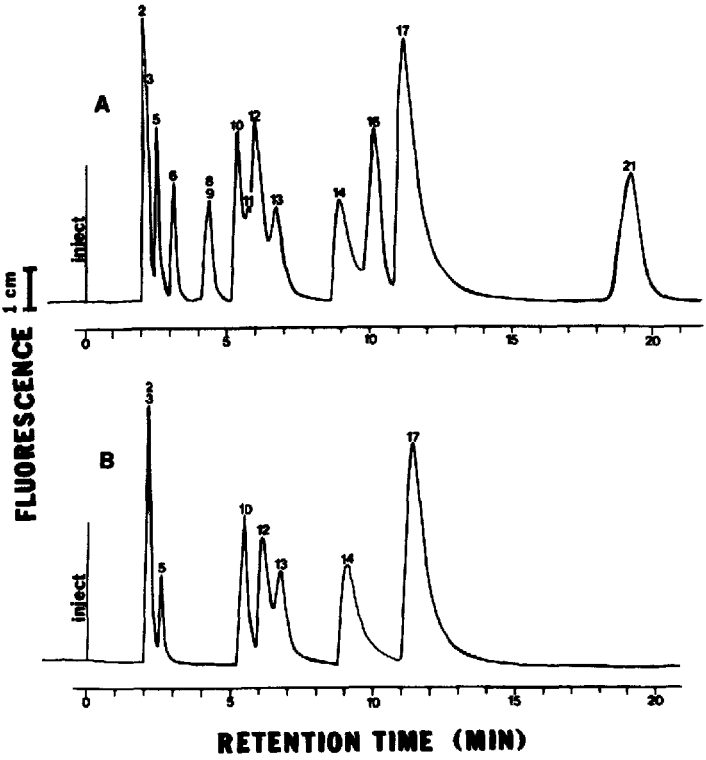

Fig 1 (A) Chromatogram resulting from the injection of 150 ng of each indole compound indicated by number as referenced in Table I, using chromatographic conditions as indicated in the text (B) Chromatogram of the formic acid eluent of weak cation-exchange precolumns after application of the same mixture of indole compounds as indicated in A to Bio-Rex 70 precolumns See text for details

Recovery from organic extraction

Extractions of TA using methylene chloride, ethanol or butanol were found to coextract large amounts of lipids which we were not able to purify without further extraction using an additional solvent system. Ethyl acetate, which is less polar than either methylene chloride or butanol, was found to extract TA well from homogenates of very basic pH where a greater percentage of TA is found in its base form (non-ionized) and where TA is less soluble in aqueous solutions.

A mixture of 20% diethyl ether in butanol has been shown to extract 5-HT even more effectively than the more common use of butanol alone [24] However, our results indicate that the combination of ethyl acetate and diethyl ether, butanol alone or diethyl ether alone were approximately equal in their ability to extract TA from homogenized tissue at a high pH (Table II) Saturation of the buffer with sodium chloride was necessary to prevent emulsification when using either butanol or diethyl ether alone (Table II), which then resulted in a slightly decreased extraction efficiency The combination of butanol and diethyl ether also resulted in the extraction of large amounts of tissue residue Based on these results, ethyl acetate was selected as the organic solvent for the extraction of TA owing to its higher rate of evaporation and lesser tendency to extract residual compounds.

TABLE II

ABILITY OF VARIOUS SOLVENTS TO EXTRACT TRYPTAMINE FROM BRAIN HOMOGENATE

Rat brain (1 g) plus 100 ng of standard TA was homogenized in 2 ml of 0 77 *M* sodium phosphate buffer (pH 10) The extraction was done with 6 ml of organic solvent Values of TA are based on comparison to controls containing tissue only

Organic solvent	Phosphate buffer	Percentage TA extracted*
Ethyl acetate	No sodium chloride added	91 3
Ethyl acetate	Saturated with sodium chloride	63 2
Butanol	No sodium chloride added	91.0
Butanol	Saturated with sodium chloride	86 7
Diethyl ether	Saturated with sodium chloride	63 6
Ethyl acetate—diethyl ether (50 50)	Saturated with sodium chloride	66 0

*Each value represents the mean value obtained from three experiments

While 5-hydroxyindoleacetic acid and indoleacetic acid are found in high concentrations in the brain, during extraction of TA with ethyl acetate at a high pH these acidic substances remain in the aqueous solution However, they are extracted into ethyl acetate from the buffer at pH 2 When determination of acid metabolites is desired, the pellet can be saved for further extraction with the organic solvent at pH 2.

Precolumn purification

The use of a weak cation-exchange resin resulted in the complete separation of 5-hydroxytryptophol, tryptophol and 5-methoxytryptophol from the remaining substances which were retained on the column (Fig. 1) These compounds are alcoholic metabolites of 5-HT, TA and 5-methoxytryptamine, respectively 5-Hydroxytryptophol has a retention time similar to TA while tryptophol and 5-methoxytryptophol have much longer retention times. Removal of these latter compounds with extremely long retention times allowed us to decrease the time between injections on the chromatograph N-Acetylated substances such as NA-5-HT (a melatonin precursor) and melatonin are also removed from the column during the initial washes (Fig. 1) This is important

TABLE III

ELUTION OF TRYPTAMINE FROM BIO-REX 70 USING VARIOUS ACIDS

Elution done according to the procedure described in Experimental, using 100 μg of tryptamine added to redissolved tissue extract to calculate recoveries

Acid	Percentage recovery in 4 ml	*n*
2 *M* Formic acid	97 9	12
2 *M* Hydrochloric acid	89 1	2
2 *M* Acetic acid	41 9	2
2 *M* Boric acid	0 0	2
2 *M* Formic acid—2 *M* hydrochloric acid (85 15)	63 8	4

as NA-5-HT has a retention time very similar to that of TA using the chromatographic conditions appropriate to maximize the peak for TA

Compounds eluted with a strong acid from the weak cation-exchange resin in addition to TA include 5-HT, 5-methoxytryptamine, 5-methyltryptamine, N-methyltryptamine and the tryptolines (Fig. 1B) After testing various acids, 2 *M* formic acid was found to be the most effective eluent of TA from the resin, resulting in a total recovery of 97 9% of the TA from the precolumn (Table III)

Total recovery

Based on the results obtained, a working extraction procedure was developed as described above in Experimental. After addition of 5 ng to 100 μg of TA to aliquots of homogenized brain tissue, followed by the entire extraction procedure and precolumn clean-up, the average total recovery (± S D) was found to be 68 9 ± 23% Use of a roto-evaporator for lyophilization of samples rather than evaporation of samples resulted in recoveries as high as 89 9 ± 4 1%

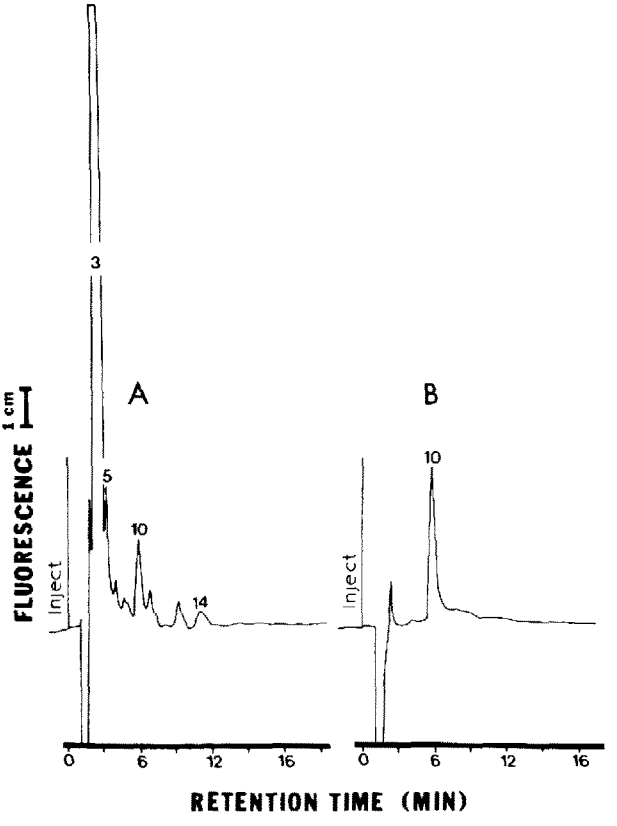

Fig 2 (A) Chromatogram of a typical final product of an extraction of tryptamine (TA) from rat brain Quantitation of the TA peak (10) indicates a value of 0 62 ng of TA per g of tissue (B) Chromatogram of a smaller and more dilute sample of extract from the brain of a rat which was pretreated with PCPA, tranylcypromine and *l*-tryptophan prior to sacrifice (as indicated in detail in the text) Sample injected in B was diluted 1 10 in mobile phase prior to injection and the volume injected was only 20% of that indicated in A Quantitation of TA in B indicates a concentration of 95 ng of TA per g tissue

The loss of TA when using the Savant Speed Vac evaporator appeared to result from warming the tubes (45°C) during the 4–5 h evaporation. This was subsequently prevented by addition of 20 μl of 10 mg/ml of ascorbic acid to the samples prior to evaporation, increasing the recovery to values typically greater than 80%.

Tissue concentrations

Using the procedure described above, the average concentration of TA in the whole rat brain was found to be 0.60 ± 0.22 ng/g (n = 12), uncorrected for the total recovery. A typical chromatogram obtained from such an extraction is shown in Fig. 2A. The concentration of TA obtained using our extraction and HPLC analysis is in close agreement with previously published values obtained using GC–MS analysis. A recently reported method [22] for the extraction and fluorometric analysis of TA in mouse brain using HPLC reported detection of TA in brain extracts only after injection of mice with 50 mg/kg TA intraperitoneally (i.p.) 2 min prior to sacrifice. The present method appears to be more sensitive as a result of the lower excitation wavelength used in the HPLC detector and also because of an alternative method of extraction and concentration of the endogenous TA.

Specific pharmacological manipulations of TA have been previously reported to alter its concentration in the brain. Treatment with 200 mg/kg PCPA i.p. daily for three days, 25 mg/kg tranylcypromine i.p. at 90 min and 100 mg/kg *l*-tryptophan i.p. at 60 min was found to increase the concentration of TA to more than 100 times its control value (Fig. 2B). This confirms proportionate increases after similar drug combinations [10, 11].

Confirmation of peak purity

We initially confirmed the purity of the endogenously obtained TA peak using manipulation of peak retention times after changes in the concentration of methanol in the HPLC mobile phase as well as by pharmacologically manipulating the concentration of endogenously formed TA using drugs previously reported to effect TA. We also used the following three approaches to eliminate the possibility that the endogenously formed TA peak merely reflects an endogenously occurring, non-tryptaminergic compound.

(1) The TA peak, resulting from the extraction and analysis of brain tissue, as described above, was collected from the μBondapak C_{18} column, dried, resuspended in 100 μl of mobile phase and reinjected in a Nova-Pak C_{18} reversed-phase column (5 μm bead size) having slightly different retention characteristics than the μBondapak column. A fluorescence peak with a retention time of 8.4 min resulted, which coincided exactly with the retention time of TA standards. Quantitation of the peak indicated that it contained an average of 58.26% (n = 3) of the original TA measured using the μBondapak column, which did not differ from the recovery of TA standards collected and processed in an identical fashion (44.6%, n = 3).

(2) Endogenous and standard TA peaks were collected from the HPLC column, dried and acetylated by reaction with acetic anhydride, which is only capable of acetylating the primary amine group on TA and other compounds. The product of this reaction with TA, NATA, was found to have a retention

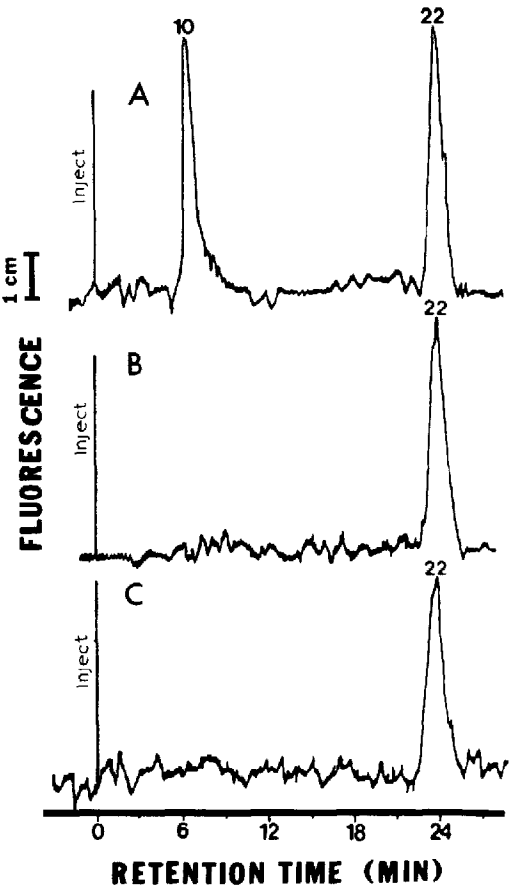

Fig 3 (A) Chromatogram resulting from the injection of 1 ng of tryptamine (TA) standard plus the product of acetylation of 1 ng of TA standard (B) Injection of the acetylated product of a 3-ng TA standard peak collected from the HPLC column (C) Injection of the product of acetylation of a 3-ng endogenous TA peak collected from an HPLC sample of brain extract (extraction as described in text)

time of 25 7 min, coincident with the reaction product of both the TA standards as well as the endogenously formed TA collected from the HPLC column (Fig 3) The average recovery of the NATA product for TA standards was 30%, which did not differ significantly from the average recovery of 25% (n = 6) of NATA formed from the TA peaks resulting from endogenous TA extracts.

(3) MS analysis of endogenous TA peaks must be done on derivatized samples as direct-probe injections of TA are not detectable using less than 200 ng of TA Derivatization of TA with TFAA, which first requires acetylation of TA, allowed us to inject the product into a capillary GC column for MS analysis

The collected and dried peaks of TA standards as well as from tissue extracts were initially acetylated and then derivatized with TFAA following the technique previously described by Warsh et al. [16] The resulting compound was determined to be N-acetyl-1-trifluoroacetyltryptamine using SIM with a

spectrum at *m/z* 115, 154, 183 and 280. This indicates that the original substance present in the collected peaks, and thus in the tissue extract, had the same chemical structure TA standards and the substance obtained from brain extract produce the same derivatized compound, confirming that the fluorescent peak measured by HPLC is in fact TA

In summary, the present study outlines an economical method for the extraction and analysis of TA from CNS tissue using an isocratic HPLC system. The high recovery of TA during the extraction and the use of an excitation wavelength of 220 rather than 280 nm provide the assay with a sensitivity in the same order of magnitude as that achieved with GC—MS

ACKNOWLEDGEMENTS

The authors wish to thank Ms Suzanne Weithers for her excellent technical assistance and suggestions during the development of this assay This research was supported by NIH Grant NS17407

REFERENCES

1 S R Philips, D A Durden and A A Boulton, Can J Biochem , 52 (1974) 448
2 A A Boulton and G B Baker, J Neurochem , 25 (1975) 447
3 A A Boulton and R S G Jones, Life Sci , 27 (1980) 1849
4 K J Kellar and C S Cascio, Eur J Pharmacol , 78 (1982) 475
5 P.L Wood, C Pilapil, F LaFaille, N P V Nair and R A Glennon, Arch Int Pharmacodyn , 268 (1984) 194
6 S M Hess and S Undenfriend, J Pharmacol Exp Ther , 127 (1959) 175
7 D Eccleston, G W Ashcroft, T B B Crawford and R Loose, J Neurochem , 13 (1966) 93
8 G W Ashcroft, D Eccleston and T B Crowford, J Neurochem , 12 (1965) 483
9 J M Saavedra and J Axelrod, J Pharmacol Exp Ther , 182 (1972) 363
10 J M Saavedra and J Axelrod, Science, 175 (1973) 1365
11 S R Snodgrass and A S Horn, J Neurochem , 21 (1973) 687
12 C A Marsden and G Curzon, Psychopharmacology, 57 (1978) 71
13 A A Boulton, Adv Biochem Psychopharmacol , 15 (1976) 57
14 S A Barker, R E Harrison, G B Brown and S T Christian, Biochem Biophys Res Commun , 87 (1979) 146
15 F Artigas, C Sunol, J M Tussel, E Martinez and E Gelpi, Biomed Mass Spectrom , 11 (1984) 142
16 J J Warsh, D D Godse, H C Staneer, P W Chan and D V Coscina, Biochem Med , 18 (1077) 10
17 J J Pisana, Clin Chim Acta, 5 (1960) 406
18 A Bertler, A Carlson and E Rosengren, Acta Physiol Scand , 44 (1958) 273
19 A Bertler, Acta Physiol Scand , 51 (1961) 75
20 J A Oates, Methods Med Res , 9 (1961) 169
21 V F Kemerer, K M Lichtenfeld and T R Koch, Clin Chim Acta, 92 (1979) 81
22 J Yamada, Y Sugimoto and K Horisaka, J Chromatogr , 311 (1984) 385
23 A M Krstulovic and C Matzura, J Chromatogr , 163 (1979) 72
24 Y Maruyama and A E Takemori, Biochem Pharmacol , 20 (1970) 1833