Journal of Chromatography, 336 (1984) 259—269
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2328

3-METHOXY-4-HYDROXYPHENYLGLYCOL, 5-HYDROXYINDOLEACETIC ACID, AND HOMOVANILLIC ACID IN HUMAN CEREBROSPINAL FLUID

STORAGE AND MEASUREMENT BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND COULOMETRIC DETECTION USING 3-METHOXY-4-HYDROXYPHENYL-LACTIC ACID AS AN INTERNAL STANDARD

MARTIN A. JAVORS*, CHARLES L. BOWDEN and JAMES W. MAAS

Departments of Psychiatry and Pharmacology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284 (U.S.A.)

(First received May 8th, 1984; revised manuscript received August 9th, 1984)

SUMMARY

To simultaneously measure 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5HIAA), and homovanillic acid (HVA) in human cerebrospinal fluid (CSF), we used an acetonitrile protein precipitation, reversed-phase high-performance liquid chromatography with coulometric detection, and 3-methoxy-4-hydroxyphenyllactic acid (MHPLA) as an internal standard for all three metabolites. MHPG, 5HIAA, HVA, and MHPLA were stable for one month when stored in CSF at −70°C. Three determinations were made in triplicate for each of seven subjects over a 30-day storage period and the coefficients of variation within subject for these determinations ranged from 0.075 to 0.165 for MHPG, 0.045 to 0.148 for 5HIAA and 0.053 to 0.181 for HVA. Means and standard deviations of CSF concentrations were 10.7 ± 3.0 ng/ml for MHPG, 22.4 ± 9.9 ng/ml for 5HIAA, and 39.9 ± 21.4 ng/ml for HVA. This method provides simple sample preparation, sensitivity, and cost advantages, as well as simultaneous extraction and quantitation of MHPG, 5HIAA, and HVA using an internal standard.

INTRODUCTION

3-Methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA) are metabolites of the central nervous systems (CNS) monoamine neurotransmitters norepinephrine (NE), dopamine (DA), and serotonin (5HT) [1—6]. The concentrations of these metabolites in cerebrospinal fluid (CSF) may reflect the activity of their relative neutrotransmitter systems in the central nervous system [7, 8]. Levels

0378-4347/84/$03.00

of these amine metabolites have been measured in patients with delirium tremens [9], senile dementia of Alzheimer type [10], sleep apnea syndrome [11], schizophrenia [12], depression and suicidal behavior [13], and Parkinson's disease [14]. The biogenic amine hypothesis of affective disorders posits a functional deficit in CNS monoamine neurotransmitters, the precursors of these metabolites [15—19]. Since intensive study of biological factors in affective disorders is a major part of current research, we wanted to develop a simple and inexpensive, yet sensitive high-performance liquid chromatographic (HPLC) assay to measure the concentrations of MHPG, 5HIAA, and HVA in human CSF.

MHPG, 5HIAA, and HVA have been measured by fluorometry [20—23], radioenzymatic assay [24], radioimmunoassay [25, 26], gas chromatography (GC) with electron-capture detection (ECD) [1, 23, 27—35], GC with mass spectrophotometric (MS) detection [6, 36—49], and by HPLC with amperometric electrochemical detection [50—59]. The earlier-developed methods, fluorometric, radioenzymatic, and GC—ECD, were not as sensitive as more recent methods and required larger amounts of CSF. Radioimmunoassay methods are relatively new for these CNS metabolites. They require derivatization of the compounds and they are sensitive in the picogram range; however, they are not being widely used at this time and have not been applied to CSF measurements. GC—MS is perhaps the most widely used method in recent years. This method is sufficiently sensitive and very specific; however, it requires equipment that is expensive both to purchase and maintain. In addition, the metabolites must be derivatized and all three metabolites cannot be quantitated simultaneously.

Assay methods for MHPG, 5HIAA, and HVA by HPLC utilizing amperometric detection have been used recently by several investigators with excellent results [55—59]. The equipment is less expensive to purchase and less troublesome to operate than that used in previous methods. The HPLC methods have included simple sample preparation and sensitivity in the sub-nanogram range.

The use of the coulometric electrochemical detector with two detector cells in a series provides sensitivity for MHPG, 5HIAA, and HVA in the low picogram range, and has the advantage of measuring compounds at two voltage settings. We here describe an enhancement of HPLC methodology for quantitation of MHPG, 5HIAA, and HVA in human CSF which combines reversed-phase HPLC with coulometric electrochemical detection. This paper is based in part on a presentation at the 5th International Catecholamine Symposium in Goteborg, Sweden [57].

EXPERIMENTAL

Materials

MHPG hemipiperazine, HVA, 5HIAA, and 3-methoxy-4-hydroxyphenyllactic acid (MHPLA) were purchased from Sigma (St. Louis, MO, U.S.A.). Sodium citrate, HPLC-grade phosphoric acid, and HPLC-grade methanol were purchased from Fisher (Pittsburgh, PA, U.S.A.). Nylon-66 filters (0.2 and 0.45 μm) were purchased from EM Science (Gibbstown, NJ, U.S.A.). The Ultrasphere-IP (C_{18}, 5 μm particle size) column was purchased from Beckman (Fullerton, CA, U.S.A.).

Tissue preparation

CSF was collected from the lumbar region of the spinal cord and was immediately placed on ice at 0—4°C [60]. MHPLA, the internal standard (20 ng/ml) was added, then the CSF was stored at —70°C in a capped polypropylene test tube until processing.

On the day of the assay, we thawed the CSF, then added 1 ml of acetonitrile to 200 μl of lumbar CSF in borosilicate test tubes. The samples were vortexed vigorously for 15 sec, then centrifuged at 2700 *g* for 15 min. This step removes approx. 99.8% of the protein in the CSF. Of the supernatant 1 ml was carefully aspirated and dried to residue under nitrogen at 40°C. This sample residue could be stored in the refrigerator at 0—4°C for 24 h, or at —70°C indefinitely, with no degradation of the metabolites.

Next we redissolved the residue in 200 μl of mobile phase, degassed the sample under house vacuum for 5 min, then injected 20 μl into the chromatograph for quantitation of MHPG, 5HIAA, HVA, and MHPLA. In these experiments, each sample was prepared immediately before injection because at pH 5 the 5HIAA is unstable in light. The 5HIAA peak in the external standard solution decreased approx. 25% at pH 5 during an 8-h period in room light. When the standard solution was protected from light, the degradation of 5HIAA did not occur. The MHPG, HVA, and MHPLA were stable at pH 5 in the light over an 8-h period.

High-performance liquid chromatography

The HPLC apparatus consisted of a Beckman Model 110A single-piston pump, a Bio-Rad Labs. Bio-Sil ODS-10 pre-column, an Altex Model 210 injector valve, a Beckman Ultrasphere-IP (250 mm × 4.1 mm I.D.), 5-μm C_{18} column, and an Environmental Systems Associates (ESA) coulometric electrochemical detector.

The isocratic mobile phase usually consisted of 4% (v/v) HPLC-grade methanol and 96% of a solution containing 75 m*M* sodium citrate and 78 m*M* phosphoric acid, pH 5.25 (see legends to figures for individual experiments). The sodium citrate—phosphoric acid solution was prepared with deionized distilled water by dissolving 75 m*M* sodium citrate, then adjusting the pH to 5.25 with phosphoric acid. As recommended by ESA, the citrate—phosphate buffer was filtered consecutively through Rainen Nylon-66 filters of 0.45 μm, then 0.2 μm pore size. The methanol was then added to the citrate—phosphate buffer and the solution was degassed for at least 5 min under house vacuum.

The ESA coulometric detector has two graphite detector cells. The mobile phase and the samples flow through these cells. At the appropriate voltage setting, 100% of an electroactive substance will be reacted as it passes through the cell. The voltage can be different for each cell and, therefore, some selectivity can result. For these experiments, detector 1 (D1) voltage was 0.23 V and detector 2 (D2) voltage was 0.40 V. 5HIAA was oxidized by D1 and MHPG, MHPLA, and HVA were oxidized by D2 (see Fig. 1).

Current—voltage curve

The current—voltage (C—V) curve was obtained by injecting several identical external standard and CSF samples into the chromatograph at different voltage

settings and measuring the current generated, then graphing the voltage (abscissa) in V versus the current (ordinate) as a fraction of the maximum current. The C—V curve is dependent on the mobile phase and provides good qualitative evidence that each peak is the result of one compound since very few compounds have the same C—V curve.

Calculation of CSF concentration

Peak heights were measured directly from a chart recorder. MHPLA in the CSF sample was added exogenously, and the metabolite extraction efficiency was calculated from data in Fig. 2.

$$\frac{\left[\frac{\text{metabolite peak height}}{\text{MHPLA peak height}}\right]_{\text{sample}}}{\left[\frac{\text{metabolite peak height}}{\text{MHPLA peak height}}\right]_{\substack{\text{external}\\ \text{standard}}}} \times [\text{MHPLA}]_{\text{sample}} \div \begin{matrix}\text{metabolite}\\ \text{extraction}\\ \text{efficiency}\\ \text{as a fraction}\end{matrix} = [\text{metabolite}]_{\text{CSF}}$$

RESULTS

Fig. 1A is the chromatogram of a 20-μl injection containing 400 pg each of MHPG, 5HIAA, HVA, and MHPLA as external standards. All four compounds eluted as baseline-to-baseline peaks within 25 min of the injection. At a full-scale detector range of 50 nA, approx. 100 pg of each of these compounds produced at least a 10% of page deflection. The 5HIAA peak (peak 3) appeared on both detectors because 100% of the 5HIAA was not oxidized by D1 at 0.23 V. We used the D1 peak height for calculations because it is directly reflective of the 5HIAA concentration. The ratios of the metabolite peaks to the MHPLA (internal standard) peak were very consistent from injection to injection and from preparation to preparation of external standard. When 400 pg each of MHPG, 5HIAA, HVA, and MHPLA were injected into the chromatograph, the ratios of external standard peak heights for MHPG/MHPLA, 5HIAA/MHPLA, and HVA/MHPLA were 1.37 ± 0.075 (S.D., n = 28), 2.01 ± 0.10 (S.D., n = 11), and 0.868 ± 0.060 (S.D., n = 28), respectively. These values were used in the calculation of the CSF concentrations of MHPG, 5HIAA, and HVA (see Fig. 2).

Fig. 1B is a chromatogram from a pooled CSF sample. Given that this was a biological sample, other peaks than those seen in Fig. 1A are noted; however, the peaks for MHPG, 5HIAA, HVA, and MHPLA are present and well defined based on retention time.

To determine the recovery of MHPG, 5HIAA, HVA, and MHPLA in our sample preparation procedure, we pooled equal amounts of CSF samples from four depressed patients and spiked six aliquots with 20 ng/ml MHPLA and 0—50 ng/ml MHPG, 5HIAA, and HVA. We then processed the samples as explained in Experimental and determined the concentrations of metabolites and internal standard in each of the six aliquots. As seen in Fig. 2, the R value (see legend to Fig. 2) of MHPG, 5HIAA, or HVA was directly proportional to the CSF concentration. Squared correlation coefficients (r^2) for the linear regressions were 0.996 for MHPG ($p < 0.0001$), 0.999 for 5HIAA ($p <$

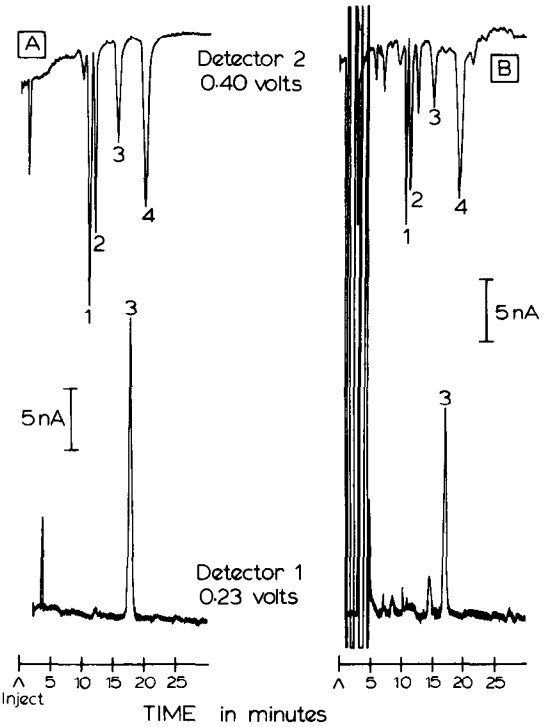

Fig. 1. (A) Chromatogram of MHPG, 5HIAA, HVA, and MHPLA standards. A 20-μl aliquot of sample containing 400 pg each of MHPG, 5HIAA, HVA, and MHPLA dissolved in mobile phase was injected into the HPLC system described in Experimental. Peaks: 1 = MHPG; 2 = MHPLA; 3 = 5HIAA; and 4 = HVA. 5HIAA is not completely oxidized by D1, therefore, a peak is seen on the D2 tracing. MHPG, MHPLA, and HVA are not oxidized by D1 and, therefore, these peaks appear only on the D2 tracing. (B) Chromatogram of a CSF sample. A 20-μl aliquot of a pooled CSF sample processed as explained in Experimental was injected into the chromatograph. The amount of each compound in the peaks is 311 pg for MHPG (1), 349 pg for MHPLA (2), 303 pg for 5HIAA (3), and 382 pg for HVA (4).

0.0001), and 0.994 for HVA ($p < 0.001$). This suggests that MHPLA is a valid internal standard for MHPG, 5HIAA, and HVA. The average recoveries for the four compounds were 94.6 ± 4.0% (S.D.) for MHPG, 76.8 ± 2.0% (S.D.) for 5HIAA, 94.2 ± 4.7% (S.D.) for HVA, and 104 ± 6.4% (S.D.) for MHPLA, the internal standard. The loss of 5HIAA occurs in the deproteinization step, not the evaporation step. These recoveries were used to calculate CSF concentrations of the metabolites.

To verify the metabolite peaks on the chromatogram of the CSF sample, we ran the CSF sample and an external standard sample at several different voltages to obtain the current—voltage curves seen in Fig. 3. The MHPG, 5HIAA, HVA, and MHPLA peak heights in standard and CSF samples increased to a maximum with voltage increases in the range 0.08—0.40 V. The fact that the curves for each compound in CSF and external standard samples were superimposable suggests that each peak was the result of a single compound.

Infrequently, a contaminating peak interfered with the accurate measurement of a metabolite peak and necessitated a minor change in the mobile

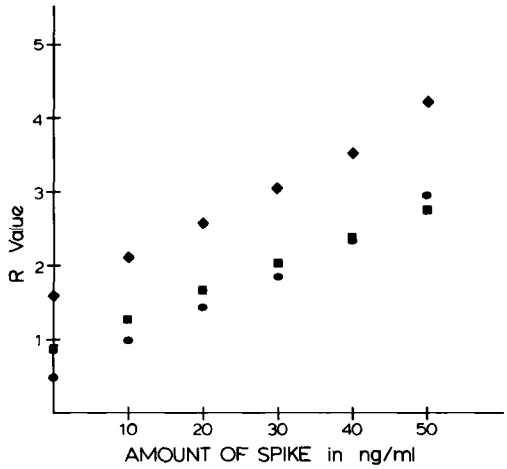

Fig. 2. Graph relating R value of MHPG, 5HIAA, and HVA to amount of spike in CSF. The R value of a compound is the ratio of each metabolite peak height to MHPLA peak height in the CSF sample divided by the same ratio for 400 pg each of the external standards. The R value for a specific compound multiplied by the concentration of the internal standard added to the CSF sample is equal to the concentration of specific compound in CSF. The abscissa is the amount of metabolite added to the CSF sample as a spike. Each of six identical pooled CSF samples was spiked with 20 ng/ml of the internal standard MHPLA and with 0, 10, 20, 30, 40 and 50 ng/ml each of MHPG, 5HIAA, and HVA. These samples were processed as described in Experimental. A 20-μl aliquot of each of the final samples was injected into the chromatograph and R values calculated from the peak height ratios in CSF and external standard injections. ●, MHPG; ■, 5HIAA; ◆, HVA.

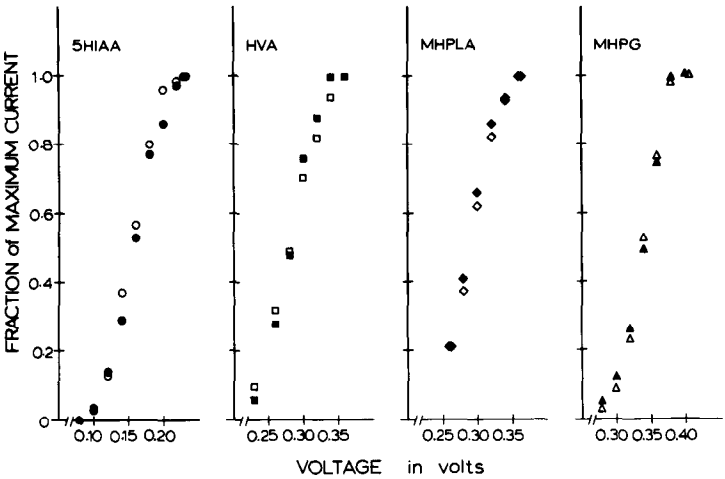

Fig. 3. Current—voltage curves for MHPG, 5HIAA, HVA, and MHPLA in external standard and CSF sample injections. The abscissa is the voltage applied to the detector cell. The ordinate is the ratio of a metabolite peak height at a given voltage to the maximum peak height. The standard solution contained 400 pg each of MHPG, 5HIAA, HVA, and MHPLA. The pooled CSF sample, spiked with only MHPLA, was prepared as explained in Experimental. The same external standard and CSF sample solutions were used throughout the experiment. Open symbols, CSF sample; closed symbols, external standards.

phase. The pH in the range 5.0—6.5 had no effect on the retention time of MHPG, but had a dramatic effect on the retention times of MHPLA, 5HIAA, and HVA. Changes of pH can be used to move the peaks in relationship to each other. Also, organic-strength changes in the mobile phase significantly altered the retention times of the MHPG, 5HIAA, HVA, and MHPLA peaks, but their relative positions on the chromatogram did not change. Ionic strength of the mobile phase had no effect on retention time of the compounds for total mobile phase concentrations of citrate and phosphate up to 200 m*M*.

To establish that the metabolites and the internal standard were stable in the CSF while stored at —70° C, we added 20 ng/ml MHPLA to freshly drawn CSF from seven subjects, then divided each subject's sample into several aliquots. Metabolite measurements were performed in triplicate with one aliquot, then the other aliquots were stored at —70° C. At 14- and 28-day intervals, aliquots were thawed and metabolite measurements were made in triplicate. There were no statistically significant differences within subject for values obtained at days 0, 14, and 28, showing that the compounds are stable when stored in CSF at —70° C. The mean coefficients of variation (standard deviation/mean) for each compound for nine determinations for each of seven subjects ranged from 0.045 to 0.181 (see Table I). These low coefficients of variation for nine determinations also show that the sample preparation was consistent.

Also presented in Table I are the CSF concentrations for MHPG, 5HIAA, and HVA. The means and standard deviations (S.D.) for seven subjects for MHPG, 5HIAA, and HVA were 10.7 ± 3.0, 22.4 ± 9.9, and 39.9 ± 21.4 ng metabolite per ml CSF, respectively. These concentrations compare favorably with the

TABLE I

CSF CONCENTRATIONS (ng/ml) AND COEFFICIENTS OF VARIATION OF MHPG, 5HIAA, and HVA IN STORED CSF SAMPLES

MHPLA, the internal standard (20 ng/ml) was added to freshly drawn CSF from lumbar punctures, then the CSF was divided into 1-ml aliquots and stored in capped polypropylene tubes at —70° C. Measurements of metabolites in triplicate were made the day of CSF drawing, then in approximately two-week intervals for two more measurements. The CSF concentrations expressed as ng/ml (± S.D.) for each metabolite for each subject are shown, and the means for seven subjects are at the bottom of the table. C.V. values are also given for each compound for nine determinations in each of seven subjects.

Subject	MHPG		5HIAA		HVA	
	CSF (± S.D.)	C.V.	CSF (± S.D.)	C.V.	CSF (± S.D.)	C.V.
1	10.4 (0.56)	0.054	9.7 (1.4)	0.148	25.7 (2.6)	0.101
2	11.3 (0.86)	0.076	29.0 (1.3)	0.045	71.8 (3.8)	0.053
3	9.3 (0.59)	0.063	22.6 (1.2)	0.054	54.8 (4.5)	0.082
4	11.8 (0.78)	0.067	19.2 (1.3)	0.068	55.9 (4.3)	0.078
5	7.5 (0.67)	0.090	8.0 (0.63)	0.078	12.4 (2.1)	0.170
6	7.8 (0.53)	0.068	14.4 (1.0)	0.069	35.3 (3.3)	0.093
7	16.5 (1.6)	0.097	14.9 (1.5)	0.104	23.3 (4.2)	0.181
Mean	10.7 (3.0)		22.4 (9.9)		39.9 (21.4)	

TABLE II

CONCENTRATION OF FREE MHPG, 5HIAA, AND HVA IN HUMAN CSF DETERMINED BY VARIOUS METHODS

CSF concentrations and standard deviations for free MHPG, total MHPG, 5HIAA, and HVA were taken from the references listed in the left column of the table. The methods used to determine the concentrations are listed in the right column.

Reference	Ref. No.	MHPG (ng/ml, ± S.D.)		5HIAA (ng/ml, ± S.D.)	HVA (ng/ml, ± S.D.)	Method
		Free	Total			
Gerbode and Bowers (1968)	21			34.0 ± 15	40 ± 29	Fluorometry
Gottfries et al. (1969)	20			40.0 ± 10	60 ± 30	Fluorometry
Wilk et al. (1972)	23			29.0	18.0	Fluorometry
Schanberg et al. (1968)	1	50—200				GC—ECD
Gordon and Oliver (1971)	27		15.1 ± 7.7	29.0	37.0	GC—ECD
Wilk et al. (1971)	28		22.0 ± 7.0			GC—ECD
Bond (1972)	30	18.1 ± 5.2	41.1 ± 18.4			GC—ECD
Sjoquist and Anggard (1972)	32				83.7 ± 5.9	GC—ECD
Wilk et al. (1972)	23		15.0			GC—ECD
Chase et al. (1973)	33	10 ± 1	14.0 ± 1.4	28.0 ± 1.3	30 ± 2.8	GC—ECD
O'Keeffe and Brooksbank (1973)	31		11.0 ± 2.9			GC—ECD
Bertilsson et al. (1972)	39			22.5 ± 9.7		GC—MS
Bertilsson (1973)	40	12.6 ± 0.88				GC—MS
Swahn et al. (1976)	42	6.99		32.5	61.9	GC—MS
Karoum et al. (1977)	45	11.0 ± 1.2	12.9			GC—MS
Jimerson et al. (1981)	38		8.0			GC—MS
Kopin et al. (1983)	49	9.0				GC—MS
Koslow et al. (1983)	36	7.97 ± 1.55		21.2 ± 5.7	41.9 ± 13.7	GC—MS
Sjoquist and Anggard (1972)	32	13.4 ± 1.1				GC—MS
Langlais et al. (1980)	56	8.0				HPLC
Tune et al. (1980)	55	4.9 ± 0.8		22.1 ± 2.1	38.4 ± 3	HPLC
Semerdjian-Rouquier et al. (1981)	58			16.7 ± 2.0	21.0 ± 5	HPLC
Van Woert et al. (1982)	59	8.0				HPLC
Javors et al. (1983)	57	11.3 ± 1.3		17.9 ± 2.5	45.0 ± 6.4	HPLC

determinations in Table II that were made in several other laboratories with various procedures.

DISCUSSION

Our goal was to develop a procedure to collect and store lumbar CSF, then to simultaneously determine MHPG, 5HIAA, and HVA concentrations in these samples. The collection of the CSF was done with a method that has worked well in our studies with infrequent and mild side-effects for the subjects [60].

The internal standard, MHPLA (Fig. 4), has structural characteristics similar to MHPG and HVA. MHPLA also has approximately the same electrochemical sensitivity as all three metabolites. As seen in Fig. 2, MHPLA was extracted from CSF in direct proportion to MHPG and HVA, as well as 5HIAA, and, therefore, was used as an internal standard for all three compounds.

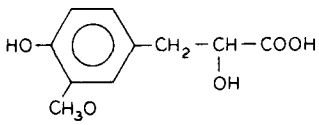

Fig. 4. Chemical structure of MHPLA.

The straightforward sample preparation, which required no derivatization, and the isocratic mobile phase allowed simultaneous extraction and quantitation of the three metabolites of interest. This is a definite advantage in sample determination time.

MHPG, 5HIAA, HVA, and MHPLA were stable for one month when stored at −70°C in capped polypropylene tubes (see Table I). For at least three separate determinations on each of three separate days, two weeks apart, the coefficients of variation for CSF concentrations of MHPG, 5HIAA, and HVA ranged between 0.045 and 0.181. Furthermore, there was no trend for an individual subject's metabolite concentration to increase or decrease during the one-month storage period. The means and standard deviations (S.D.) of the CSF concentrations for the seven subjects in our study were 10.7 ± 3.0 ng/ml for MHPG, 22.4 ± 9.9 ng/ml for 5HIAA, and 39.9 ± 21.4 ng/ml for HVA. As seen in Table II, these values are in the same range as those calculated by HPLC with amperometric detection and GC—MS or GC—ECD. In fact, the values that have been determined over the last fifteen years by various methods are very consistent.

The advantages of the HPLC with coulometric detection are in relative cost, selectivity, and sensitivity. An isocratic HPLC with electrochemical detection is much less expensive to purchase and maintain than either a GC—MS or a GC—ECD system. Reversed-phase HPLC provides exceptional selectivity in the separation of low-molecular-weight compounds. MHPG, 5HIAA, HVA, and the internal standard MHPLA were easily separated by appropriate organic strength and pH of an isocratic mobile phase.

Furthermore, the coulometric detector we used has two detector cells in series which provides further selectivity. Although we have not fully utilized this advantage in this study, it is possible to set each detector at a voltage that

would oxidize a fraction of a specific compound, giving a signal in each channel. The ratio of the signal from D1 to the signal from D2 would be constant for a specific compound, and would confer more selectivity to the system. In addition, in more complex samples, as those from urine or plasma, having two detector cells set at different voltages would allow different compounds to be quantitated on D1 and D2. Finally, HPLC with coulometric detection enabled us to actually measure as little as 20 pg of MHPG, 5HIAA, HVA, and MHPLA. This sensitivity allows for more dilute samples and, therefore greater HPLC column life.

ACKNOWLEDGEMENTS

The authors thank Mr. Roger Juarez for expert technical assistance, Mr. John Morrison, ESA, and Dr. John Towell, Milwaukee VA Hospital, for invaluable discussion, as well as Terry Ryan for typing the manuscript.

REFERENCES

1 S.M. Schanberg, G.R. Breese, J.J. Schildkraut, E.K. Gordon and I.J. Kopin, Biochem. Pharmacol., 17 (1968) 2006—2008.
2 J. Glowinski, I.J. Kopin and J. Axelrod, J. Neurochem., 12 (1965) 25—30.
3 C.O. Rutledge and J. Jonason J. Pharmacol. Exp. Ther., 157 (1967) 493—502.
4 J.W. Maas and D.H. Landis, J. Pharmacol. Exp. Ther., 163 (1968) 147—162.
5 D.F. Sharman, Brit. J. Pharmacol., 36 (1969) 523—534.
6 F. Karoum, J. Moyer-Schwing, S.G. Potkin and R.J. Wyatt, Commun, Psychopharmacol., 1 (1977) 343—352.
7 J.L. Meek and N.H. Neff, J. Pharmacol. Exp. Ther., 184 (1973) 570—575.
8 J. Korf, G.K. Aghajanian and R.H. Roth, Eur. J. Pharmacol., 21 (1973) 305—310.
9 C.M. Banki and G. Molnar, Psychiatria Clinica, 14 (1981) 167—177.
10 H. Soininen, E. MacDonald, M. Rekonen and P.J. Riekkinen, Acta Neurol. Scand., 64 (1981) 101—107.
11 H. Cramer, J.-M. Warter, B. Renaud, J. Krieger, C.H.R. Marescaux and R. Hammers, J. Neurol. Neurosurg. Psychiatry, 44 (1981) 1165—1167.
12 G. Sedvall, Adv. Exp. Med. Biol., 133 (1981) 719—725.
13 M. Asberg and L. Traskman, Adv. Exp. Med. Biol., 133 (1981) 739—752.
14 J. Mendlewicz, J.-E. Vanderheyden and G. Noel, Adv. Exp. Med. Biol., 133 (1981) 753—767.
15 M. Asberg, P. Thoren, L. Traskman, L. Bertilsson and V. Ringberger, Science, 191 (1976) 478—480.
16 W.E. Bunney and J.M. Davis, Arch. Gen. Psychiatry, 13 (1965) 483—494.
17 J.W. Maas, Arch. Gen. Psychiatry, 32 (1975) 1357—1361.
18 J.J. Schildkraut, Amer. J. Psychiatry, 122 (1965) 509—522.
19 H.M. van Praag, Biol. Psychiatry, 12 (1977) 101—131.
20 C.G. Gottfries, I. Gottfries and B.E. Roos, J. Neurochem., 16 (1969) 1341—1345.
21 F.A. Gerbode and M.B. Bowers, J. Neurochem., 15 (1968) 1053—1055.
22 G.W. Ashcroft and D.F. Sharman, Brit. J. Pharmacol., 19 (1962) 153—160.
23 S. Wilk, B. Shopsin, S. Gershon and M. Suhl, Nature, 235 (1972) 440—441.
24 N.D. Vlachakis, N. Alexander, M.T. Velasquez and R.F. Maronde, Biochem. Med., 22 (1979) 323—331.
25 J.J. Puizillout and M.A. Delaage, J. Pharmacol. Exp. Ther., 217 (1981) 791—797.
26 T.K. Keeton, H. Krutzsch and W. Lovenberg, Science, 211 (1981) 586—588.
27 E.K. Gordon and J. Oliver, Clin. Chim. Acta, 35 (1971) 145—150.
28 S. Wilk, K.L. Davis and S.B. Thacker, Anal. Biochem., 39 (1971) 498—504.
29 S. Wilk, S.E. Gitlow, D.D. Clarke and D.H. Paley, Clin. Chim. Acta, 16 (1967) 403—408.

30 P.A. Bond, Biochem. Med., 6 (1972) 36—45.
31 R. O'Keeffe and B.W.L. Brooksbank, Clin. Chem., 19 (1973) 1031—1035.
32 B. Sjoquist and E. Anggard, Anal. Chem., 44 (1972) 2297—2301.
33 T.N. Chase, E.K. Gordon and L.K.Y. Ng, J. Neurochem., 21 (1973) 581—587.
34 H. Dekirmenjian and J.W. Maas, Clin. Chim. Acta., 52 (1974) 203—210.
35 A.E. Halaris, E.M. Demet and M.E. Halari, Clin. Chim. Acta, 78 (1977) 285—294.
36 S.H. Koslow, J.W. Maas, C.L. Bowden, J.M. Davis, I. Hanin and J.A. Javaid, Arch. Gen. Psychiatry, 40 (1983) 999—1010.
37 J.W. Maas, S.E. Hattox, N.M. Greene and D.H. Landis, Science, 205 (1979) 1025—1027.
38 D.C. Jimerson, J.C. Ballenger, C.R. Lake, R.M. Post, F.K. Goodwin and I.J. Kopin, Psychopharmacol. Bull., 17 (1981) 86—87.
39 L. Bertilsson, A.L. Atkinson, Jr., J.R. Althaus, A. Harfast, J.-E. Lindgren and B. Holmstedt, Anal. Chem., 44 (1972) 1434—1438.
40 L. Bertilsson, J. Chromatogr., 87 (1973) 147—153.
41 B. Sjöquist, B. Lindström and E. Änggård, J. Chromatogr., 105 (1975) 309—316.
42 C.-G. Swahn, B. Sandgarde, F.-A. Wiesel and G. Sedvall, Psychopharmacology, 48 (1976) 147—152.
43 J.J. Warsh, D.D. Godse, S.W. Cheung and P.P. Li, J. Neurochem., 36 (1981), 893—901.
44 S. Takahashi, D.D. Godse, J.J. Warsh and H.C. Stancer, Clin. Chim. Acta, 81 (1977) 183—192.
45 F. Karoum, J. Moyer-Schwing, S.G. Potkin and R.J. Wyatt, Brain Res., 125 (1977) 333—339.
46 J.W. Maas, S.E. Hattox, D.M. Martin and D.H. Landis, J. Neurochem., 32 (1979) 839—843.
47 J.W. Maas, S.E. Hattox, N.M. Greene and D.H. Landis, J. Neurochem., 34 (1980) 1547—1549.
48 J.W. Maas, S.E. Hattox, D.H. Landis and R.H. Roth, Brain Res., 118 (1976) 167—173.
49 I.J. Kopin, E.K. Gordon, D.C. Jimerson and R.J.Polinsky, Science, 219 (1983) 73—75.
50 M. Scheinin, W.-H. Chang, D.C. Jimerson and M. Linnoila, Anal. Biochem., 132 (1983) 165—170.
51 H. Ong, F. Capet-Antonini, N. Yamaguchi and D. Lamontagne, J. Chromatogr., 233 (1982) 97—105.
52 D.M. Martin, R.E. Petroski, J. Alrazi, R. Hahn, A.L.C. Pottash and M.S. Gold, Society for Neuroscience Meeting, Abstract No. 126.16, 1983, p. 431.
53 P.A. Shea and J.B. Howell, Society for Neuroscience Meeting, Abstract No. 290.15, 1983, p. 998.
54 M. Bockstaele, L. Dillen, M. Clayes and W.P. DePotter, Arch. Int. Pharmacodyn. Ther., 260 (1982) 304—305.
55 L. Tune, R. Zaczek and J.T. Coyle, Society for Neuroscience Meeting, Abstract No. 40.8, 1980, p. 108.
56 P.J. Langlais, W.J. McEntee and E.D. Bird, Clin. Chem., 26 (1980) 786—788.
57 M.A. Javors, C.L. Bowden and J.W. Maas, 5th International Catecholamine Symposium, Abstract No. 238, 1983, p. 177.
58 L. Semerdjian-Rouquier, L. Bossi and B. Scatton, J. Chromatogr., 218 (1981) 663—670.
59 M.H. Van Woert, I. Magnussen, Y.H. Lowe and E.C. Hwang, J. Histochem. Cytochem., 30 (1982) 824—827.
60 S. Secunda, S.H. Koslow, D.E. Redmond, D. Garver, T.A. Ramsey, J. Croughan, J.H. Kocsis, I. Hanin and K. Lieberman, Psychol. Med., 10 (1980) 777—793.