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DETERMINATION OF FREE 3-METHOXY-4-HYDROXYPHENYLGLYCOL WITH SEVERAL OTHER MONOAMINE METABOLITES IN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH AMPEROMETRIC DETECTION

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SUMMARY

A reversed-phase liquid chromatographic method with amperometric detection has been developed for determining free 3-methoxy-4-hydroxyphenylglycol in plasma. The method is based on a simple and rapid extraction procedure employing a small C_{18} column. Vanillyl alcohol was used as an internal standard to obtain a good reproducibility. The 3-methoxy-4-hydroxyphenylglycol concentrations measured with the present method were in reasonable agreement with recently published data using high-performance liquid chromatography with amperometric detection and gas chromatography with mass spectrometry. The additional advantage of the present assay is that it can be performed in parallel with the quantification of other monoamine metabolites in plasma.

INTRODUCTION

Changes in the activity of the brain monoaminergic system have been implicated in a number of pathological states including psychiatric and neurological disorders [1—4]. Free 3-methoxy-4-hydroxyphenylglycol (MHPG) is a principal metabolite of norepinephrine in the human brain [5, 6], and is considered to pass readily out into the blood and cerebrospinal fluid (CSF) [7]. The assessment of metabolite(s) in the CSF is difficult for ethical reasons under usual clinical settings. The measurement of free MHPG concentrations in plasma has been suggested to offer a useful index of central adrenergic activity in human subjects [5, 8], although some controversial problems still remain to be solved [9].

Gas chromatographic methods with mass spectrometry (GC—MS) [10—12],

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which have most commonly been used for the measurement of plasma MHPG concentration, require expensive apparatus and superior technique. High-performance liquid chromatographic methods with amperometric detection (HPLC—ED) have recently been shown to be useful for the determination of MHPG in urine [13, 14], brain [15, 16], and CSF [17—20]. To our knowledge, however, only two recent articles [21, 22] have described the HPLC-ED method for the determination of free MHPG concentrations in plasma. Those experimental procedures [21, 22], however, necessitated a somewhat tedious pre-extraction of MHPG from plasma. In addition, several compounds used as internal standards in the previous HPLC—ED assays [14, 18, 21] do not appear to be readily available.

This article describes an HPLC—ED method for determining free MHPG concentrations in human plasma using vanillyl alcohol as the internal standard. The method necessitates a simple one-step sample purification step on a small C_{18} column. Furthermore, the present assay method, when used in combination with the method recently reported by us [23], permits four major monoamine metabolites in human plasma to be readily profiled.

EXPERIMENTAL

Reagent

4-Hydroxy-3-methxoybenzyl alcohol (vanillyl alcohol, VA) was obtained from Aldrich (Milwaukee, WI, U.S.A.). 3-Methoxy-4-hydroxyphenylglycol (MHPG) hemipiperazine salt and other related compounds were purchased from Sigma (St. Louis, MO, U.S.A.). All other reagents were of analytical reagent grade.

Chromatography

The liquid chromatography—electrochemical detection system consisted of a Yanako Model L-4000 W pump, a 250 × 4.6 mm I.D. 7-μm Yanapak ODS-A reversed-phase column and a Model VMD-501 dual electrochemical detector with glassy carbon electrodes. All of the system was purchased from Yanagimoto (Kyoto, Japan). Applied potential was +0.7 V versus Ag/AgCl reference electrode. The mobile phase, 0.1 *M* potassium phosphate buffer (pH 4.8) containing EDTA · 2Na (10 μM) and methanol (10%), was delivered at a flow-rate of 1.1 ml/min at ambient temperature.

Extraction

Blood was collected in a tube containing 0.1% EDTA · 2Na and 0.1% sodium metabisulphite and put on ice. Plasma was separated by centrifugation at 600 *g* for 7 min at 4° C, and then stored at −80° C until analysed.

Extraction was performed under vacuum using Bond-Elut columns pre-packed with 100 mg of C_{18}-bonded silica (40 μm) in a 1-ml capacity disposable syringe (Analytichem International, Harbor City, CA, U.S.A.). The columns, which were inserted into a vacuum chamber connected with a water aspirator, were prepared by washing with 1 ml of methanol followed by 1 ml of water.

After the addition of 50 μl of a solution of VA (internal standard equivalent

to 5 ng) to 1 ml of plasma, samples were applied to and passed through the columns, followed by 0.75 ml of water to rinse off both the residual samples and easily eluted hydrophilic compounds. The adsorbed materials were eluted with 200 μl of methanol—0.1 *M* phosphate buffer (pH 4.8) mixture (40:60). Usually 20 μl of this solution were injected into the HPLC system.

Calibration curves were generated by processing authentic standard substances through the entire extraction procedure and comparing the peak heights with that of the internal standard.

RESULTS AND DISCUSSION

The principal problems associated with the determination of plasma MHPG concentrations using HPLC—ED have been concerned with the selection of an appropriate extraction procedure prior to injection into the HPLC system [13, 21, 22] and the selection of the compound used as internal standard [14, 21]. To solve the latter problem, various neutral compounds such as 4-hydroxybenzyl alcohol, 3-(*p*-hydroxyphenyl)-1-propanol, and 3-hydroxy-4-methoxybenzyl alcohol (isovanillyl alcohol) were examined in the present study. VA was chosen because of its appropriate chromatographic behaviour and sufficient detectability at the potential of 0.7 V (Fig. 1), which allowed the MHPG analysis to be sensitive and selective.

Typical chromatograms showing the separation of MHPG and VA (internal standard) in standard solution and in an extract of human plasma are shown in Figs. 1 and 2, respectively. Under the present chromatographic conditions, related basic monoamines except for serotonin which was retained longer than MHPG, were eluted within 5 min (Table I). All of the acidic and other neutral metabolites examined did not interfere with the detection of MHPG and VA. Furthermore, the pH of the mobile phase and the column temperature employed in the present assay method substantially contributed to an optimal separation from other possible interferences by endogenous substances in human plasma. Unidentified peaks, which appeared just after MHPG (Fig. 2), overlapped at temperatures above 35°C or with increasing pH. Identities of the peaks were verified by the chromatographic behaviour under varying conditions and electrochemical characteristics determined by a dual-electrode detection system, which enabled the relative magnitudes of current responses of standards and unknowns to be compared at two different potentials.

Effective and rapid extraction was accomplished with the use of a small C_{18} column. Neutral metabolites such as MHPG and VA were readily sorbed on a hydrophobic C_{18} sorbent from plasma and eluted with a methanol—buffer mixture. When the plasma sample was acidified prior to applying it to the extraction column, the bulk of interfering substances was also sorbed, resulting in a disturbance of the subsequent chromatographic analysis. Since MHPG is a fairly hydrophilic compound, the volume of water used for washing had to be set at 0.75 ml in order to remove interference(s) caused by more hydrophilic compound(s) than MHPG and to minimize the possible loss of MHPG. The use of methanol as the eluent in place of methanol—buffer mixture also led to an increase in interfering peaks. Through the entire extraction procedure in the present assay method, the majority of acidic monoamine metabolites and the

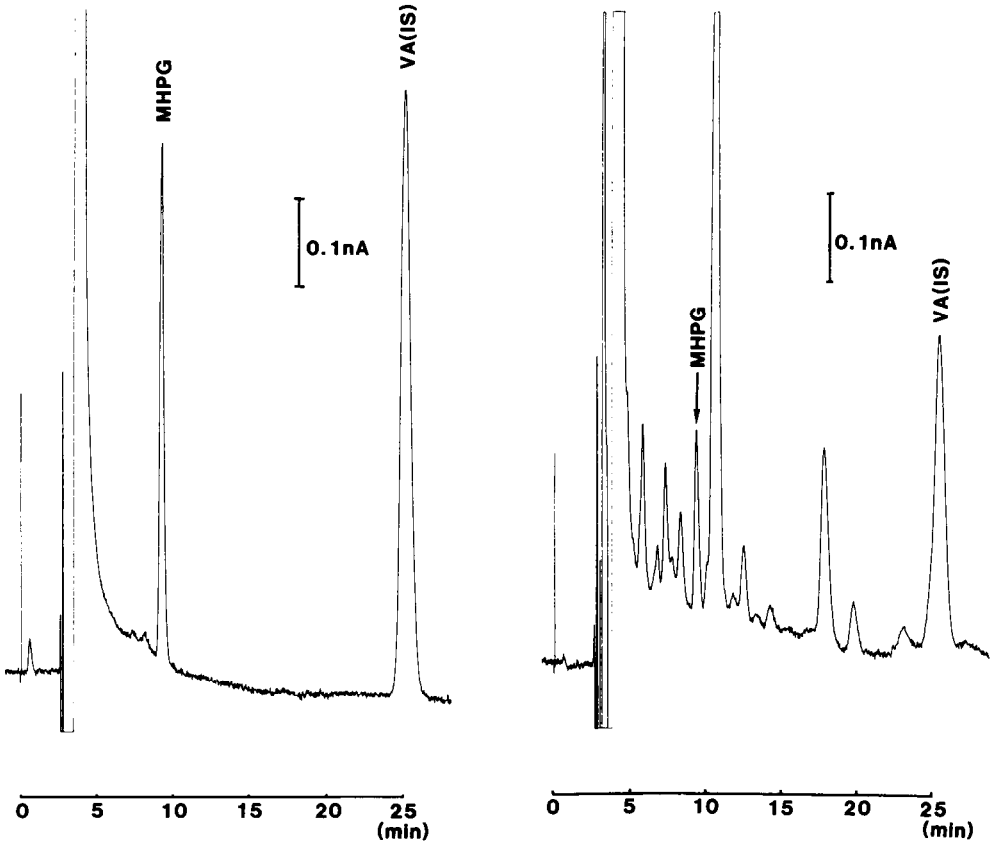

Fig. 1. Chromatogram of authentic MHPG and vanillyl alcohol (VA as internal standard, IS) in standard solution (0.8 ng each).

Fig. 2. Typical chromatogram of MHPG (3.54 ng/ml) and vanillyl alcohol (VA) in an extract from human plasma.

TABLE I

RETENTION TIMES OF SOME MONOAMINE RELATED COMPOUNDS

Compound	Retention time (min)
L-Dopa	3.80
3,4-Dihydroxyphenylglycol	4.65
Dopamine	4.67
Tyrosine	4.73
5-Hydroxytryptophan	8.18
L-Tryptophan	8.23
3,4-Dihydroxyphenylacetic acid (DOPAC)	8.54
3-Methoxy-4-hydroxyphenylglycol (MHPG)	9.24
Serotonin	10.70
5-Hydroxyindoleacetic acid (5-HIAA)	19.65
Homovanillic acid (HVA)	22.74
Vanillyl alcohol (VA)	25.08

relevant amino acids were eliminated together with many unknown interfering compounds.

The recovery rates from plasma throughout the entire procedure were around 60.88% for MHPG and 95.70% for VA (Table II). The highly sufficient reproducibility of the assay was confirmed by the coefficient of variation (C.V.) which ranged from 2.63% to 5.39% (Table II). A standard curve was run on each occasion with known amounts of MHPG added to plasma samples and extracted as described above. The linearity over the range 0.5—20 ng/ml was demonstrated by the plot of the peak height ratios of MHPG to VA (5 ng/ml) against the added amounts of MHPG. The standard curve had an r value of 0.994 and intercepted the origin ($Y = 0.145X + 0.004$, where Y is the peak height ratio and X is the concentration in ng).

TABLE II

PRECISION OF MHPG DETERMINATION FROM POOLED PLASMA AND RECOVERY OF ADDED MHPG AND VA

Compound	Concentration ± S.D. (ng/ml)	C.V. (%)	Amount added (ng)	Recovery ± S.D. (%) ($n = 5$)
MHPG				
Pool 1	6.48 ± 0.17 ($n = 5$)	2.63 (within-day)	5	57.55 ± 3.38
Pool 2	4.08 ± 0.22 ($n = 9$)	5.39 (between-day)	10	60.88 ± 3.53
Pool 3	8.25 ± 0.30 ($n = 9$)	3.64 (between-day)		
VA (internal standard)	—	—	5	95.70 ± 2.41

TABLE III

DETERMINATION OF PLASMA MHPG FROM HEALTHY HUMAN SUBJECTS

Subject number	MHPG concentration in plasma (ng/ml)
1	6.73
2	6.65
3	3.48
4	6.73
5	5.19
Mean	5.76
±S.D.	±1.28

The values for free MHPG concentrations in plasma from five healthy volunteers are given in Table III, which were found to be in agreement with the recently published data using HPLC—ED [21, 22] and GC—MS [5, 7].

Recently, we described a method for the simultaneous determination of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), metabolites of dopamine, and 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin, in human plasma by HPLC—ED [23]. The simultaneous utilization of the present and previous assay methods permits the determination of four major monoamine metabolites — MHPG, DOPAC, HVA and 5-HIAA — in plasma after the parallel extraction of MHPG and the other three metabolites from approximately equal portions of an aliquot of

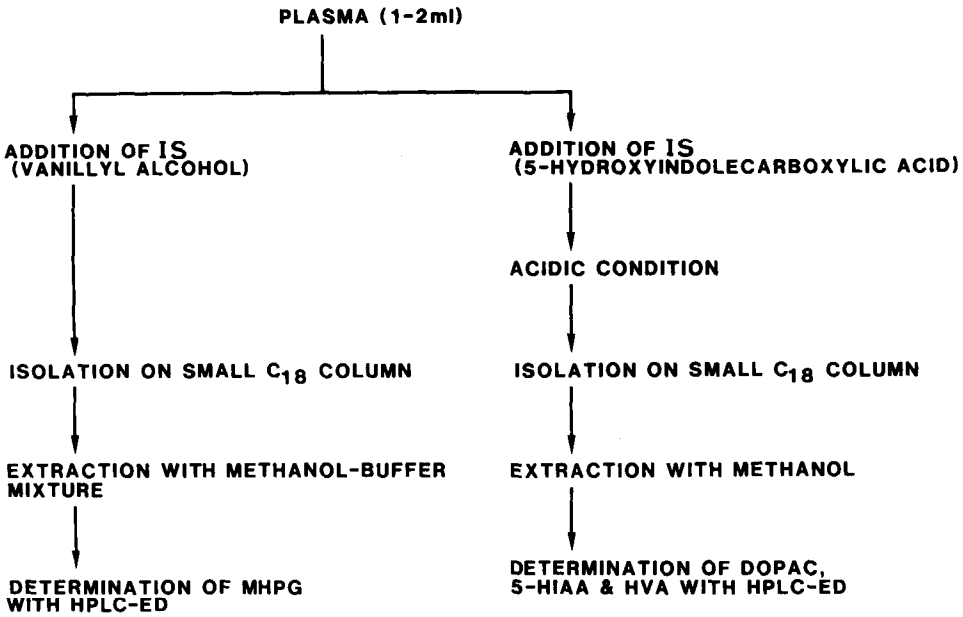

Fig. 3. Combined method for determination of plasma monoamine metabolites. Abbreviations: DOPAC = 3,4-dihydroxyphenylacetic acid; 5-HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid; MHPG = 3-methoxy-4-hydroxyphenylglycol; IS = internal standard.

plasma sample (Fig. 3). The present and previous methods are clinically applicable by employing just 500-μl aliquots of plasma. Such a combined method may further facilitate the clinical applicability of the measurement of monoamine metabolites in plasma at various pathological states [1—4] including psychiatric disorders. By using this combined method, a clinical study is underway in our laboratory; the results will be reported elsewhere.

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REFERENCES

1 B.E. Leonard, Neurochem. Int., 4 (1982) 339.
2 P. Riederer, K. Toifl and P. Kurzik, Clin. Chim. Acta, 123 (1982) 27.
3 R.F. Harvey, D.T. Fullerton, I.H. Carlson, M.H. Klin and T. Howell, Arch. Phys. Med. Rehabil., 63 (1982) 419.
4 G.N. Ko, J.D. Elsworth, R.H. Roth, B.G. Rifkin, H. Leigh and E. Redmond, Arch. Gen. Psychiatry, 40 (1983) 425.
5 J.W. Maas, S.E. Hattox, N.M. Greene and D.H. Landis, Science, 205 (1979) 1025.
6 E.M. DeMet and A.E. Halaris, Biochem. Pharmacol., 28 (1979) 3043.
7 I.J. Kopin, F.K. Goodwin, D.C. Jimerson and R.J. Polinsky, Science, 219 (1983) 73.
8 J.D. Elsworth, D.E. Redmond, Jr. and R.H. Roth, Brain Res., 235 (1982) 115.
9 I.J. Kopin, R.J. Polinsky, J.A. Oliver, I.R. Oddershede and M.H. Ebert, J. Clin. Endocrinol. Metab., 57 (1983) 632.

10 B. Sjöquist, B. Lindström and E. Änggård, J. Chromatogr., 105 (1975) 309.
11 F.A.J. Muskiet, G.T. Nagel and B.Y. Wolthers, Anal. Biochem., 109 (1980) 130.
12 D.C. Jimerson, S.P. Markey, J.A. Oliver and I.J. Kopin, Biomed. Mass Spectrom., 8 (1981) 256.
13 R. Alonso, C.J. Gibson and J. McGill, Life Sci., 29 (1981) 1689.
14 G. Santagostino, P. Frattini, S. Schinelli, M.L. Cucchi and G.L. Corona, J. Chromatogr., 233 (1982) 89.
15 A.J. Cross and M.H. Joseph, Life Sci., 28 (1981) 499.
16 J.F. Towell and V.G. Erwin, J. Chromatogr., 223 (1981) 295.
17 A.M. Krstulovic, L. Bertani-Dziedzic, S.W. Dziedzic and S.E. Gitlow, J. Chromatogr., 223 (1981) 305.
18 P. Frattini, G. Santagostine, M.L. Cucchi, G.L. Corona and S. Schinelli, Clin. Chim. Acta, 125 (1982) 97.
19 P.J Langlais, W.J. McEntee and E.D. Bird, Clin. Chem., 26 (1980) 786.
20 G.M. Anderson, J.G. Young, D.J. Cohen, B.A. Shaywitz and D.K. Batter, J. Chromatogr., 222 (1981) 112.
21 M. Scheinin, W-H. Chang, D.C. Jimerson and M. Linnoila, Anal. Biochem., 132 (1983) 165.
22 H. Ong, F. Capet-Antonini, N. Yamaguchi and D. Lamontagne, J. Chromatogr., 233 (1982) 97.
23 A. Mineghishi and T. Ishizaki, J. Chromatogr., 308 (1984) 55.