Assay for 3-Methoxy-4-hydroxyphenylethylene Glycol in Human Urine by Gas Chromatography, with Electron Capture Detection

Chul Kim¹ and Glen R. Van Loon²

We describe a gas-chromatographic method, with electron capture detection, for determining 3-methoxy-4-hydroxyphenylethylene glycol in human urine. High sensitivity (5 pg per injection), recovery (93–97%) and reproducibility (CV 4.3%) are obtained. By this method, the 24-h urinary excretion of the free compound, the sulfate conjugate, and the total in six normal male subjects averaged 0.15 (SEM 0.015), 1.09 (SEM 0.10), 1.22 (SEM 0.10), and 2.34 (SEM 0.21) mg, respectively, in three young women with amenorrhea/hirsutism, administration of dexamethasone decreased the total excretion of the compound.

Additional Keyphrases: norepinephrine metabolite and its conjugate • amenorrhea • hirsutism • dexamethasone • reference interval

Since 3-methoxy-4-hydroxy-phenylethylene glycol (MHPG) was isolated and characterized as a metabolite of norepinephrine that is normally present in urine (1), several methods have been described for its determination. Paper chromatographic separation (2, 3) has limited accuracy. Spectrometric or fluorometric measurements (4–6) are not specific and poorly sensitive. Several methods involving gas chromatography with flame ionization detection (7, 8) or electron capture detector (ECD) (9–19) have been described; the sensitivity of the former is much lower than that of the latter. Methods involving gas chromatography with mass spectrometry in determining MHPG in human cerebrospinal fluid (20–22) and serum (23) offer unique specificity and high sensitivity, but are technically complex and expensive to acquire, and the method in which stable isotope dilution is used (24) involves extraction of the urine sample on ion-exchange resin followed by isolation of MHPG-sulfate by column chromatography. A “high-performance” liquid chromatographic method (25) requires back extraction for purification before gas chromatography. The reports of gas chromatography with ECD methods describe various steps for isolating, purifying, and quantitating MHPG in urine. Absolute values of MHPG and its recovery are variable.

The present study describes a simplified method of gas chromatography with ECD for measuring MHPG in human urine with high sensitivity, analytical recovery, and reproducibility.

Materials and Methods

Human urine was stored at −20 °C without acidification until analyzed; 250 μL was mixed with 50 μL of a saturated solution of BaCl₂ and 0.3 mL of distilled water, then centrifuged at 31 500 × g for 20 min. To 0.3 mL of the supernate, 0.2 mL of acetic acid buffer (1 mol/L, pH 6.0) was added. Appropriate enzymes were used for total and conjugated MHPG: 50 μL of “Glusulase” [β-glucuronidase/sulfatase preparation from Helix pomatia, Type H-2, 120 000 Fishman units of β-D-glucuronidase (EC 3.2.1.31) per milliliter and 3270 Sigma units of aryl-sulfatase (EC 3.1.6.1) per liter, cat. no. G-0876; Sigma Chemical Co., St. Louis, MO 63178] for total MHPG; 7.5 μL of aryl-sulfatase from Helix pomatia (5 units of aryl-sulfatase per milligram, with <2% β-glucuronidase contaminant, cat. no. 102890; Boehringer Mannheim, Indianapolis, IN 46250) for the sulfate conjugate, and 2.5 mg of β-glucuronidase from bovine liver (Type B-1, 903 000 Fishman units/g, cat. no. G-0251; Sigma) for glucuronide conjugate. The samples were hydrolyzed for 20 h in a water bath at 40 °C for assay of conjugated metabolites. After hydrolysis, the samples were cooled, saturated with NaCl, centrifuged (31 500 × g, 20 min), and 0.2 mL of the supernate was collected for extraction.

For free MHPG determination, the same procedure was followed, but with no enzymatic treatment.

To 0.2 mL of sample, 4 μg of tryptophol (Aldrich Chemical Co., Milwaukee, WI 53282) was added as internal standard (14). The samples were extracted once with 1.0 mL of glass-distilled ethyl acetate, and 250 μL was transferred into 1.0-mL reaction vials. The ethyl acetate extracts were dried under reduced pressure in an evaporator. The residues were reacted directly with 25 μL of pentafluoropropionic anhydride (Pierce Chemical Co., Rockford, IL 61050) for 10 min at 70 °C. After excess pentafluoropropionic anhydride was evaporated away under dry nitrogen, the pentafluoropropionate derivative was reconstituted in 500 μL of ethyl acetate and 1 μL was injected into the gas chromatograph with a 5-μL Hamilton syringe.

We used a chromatograph (Model 5730A; Hewlett-Packard, Avondale, PA 19311) equipped with an ECD (15 mCi 63Ni) and integrator (Model 3380A, Hewlett-Packard). The gas chromatographic separation was on a 182 cm × 4 mm i.d. coated glass column containing 3% OV-17 coated on Glass Chrom Q 100–120 mesh (Chromatographic Specialties, Brockville, Ontario K6V 5W1). The column temperature was maintained isothermally at 155 °C. Injection port and detector temperature were maintained at 200 °C. The carrier gas used was a 5/95 by vol mixture of methane and argon, at a flow rate of 30 mL/min.

Known amounts (1, 2, and 4 μg of base) of authentic bis-MHPG piperazine salt (Calbiochem, San Diego, CA 92112) were added to 1-mL aliquots of a urine sample, which were taken through the entire procedure, tryptophol being added to each as an internal standard. The MHPG response obtained from an aliquot of the same urine without added MHPG was subtracted from that of each of these standard samples, and the MHPG/tryptophol response ratios were plotted vs ratios of amounts of injected MHPG/tryptophol to construct a standard curve.

Results and Discussion

MHPG and its internal standard tryptophol are clearly separated, and no interfering endogenous compounds are apparent (Figure 1).

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Figure 1. Representative chromatogram of MHPG and its internal standard tryptophol.
A. Chromatogram of MHPG and tryptophol standards in distilled water. B. Chromatogram of a urine sample, showing peak for endogenous MHPG and peak of internal standard, tryptophol.

Figure 2 shows linearity of the plot of peak area response ratio (MHPG/tryptophol) with increasing concentrations of injected MHPG (r = 0.998). The limit of detection is 5 pg per injection for either standard or urine equivalents, and the linearity of the concentration and response relation was established up to 320 pg per injection. Analytical recovery of MHPG (0.25-1.0 μg) added to unhydrolyzed urine ranged from 93 to 97%. The coefficient of variation for the assay of normal urine is 4.3%. The detector responses to different volumes of urine hydrolyzed with Glusulase are shown in Figure 3. The amount of enzyme used to hydrolyze 0.25 mL of human urine (the volume usually used in our assay) is evidently sufficient, because the detector response is linear. Determination of the relation between incubation interval and maximum hydrolysis of conjugated MHPG with Glusulase demonstrated, as discussed also by Sharpless (18), that the hydrolysis is complete by 20 h at 40 °C.

**Table 1. Concentrations of Free, Conjugated, and Total MHPG in Normal Human Urine: Present and Reported Data**

<table>
<thead>
<tr>
<th>Free</th>
<th>Sulfate</th>
<th>Glucuronide</th>
<th>Total</th>
<th>Recovery</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.21 ± 0.35</td>
<td>1.30 ± 0.18</td>
<td>1.21 ± 0.24</td>
<td>0.5 - 2.0</td>
<td>9-47%</td>
<td>Paper chromatog.</td>
<td>2</td>
</tr>
<tr>
<td>0.05 ± 0.007</td>
<td>0.85 ± 0.074</td>
<td>0.98 ± 0.084</td>
<td>0.27 ± 0.22</td>
<td>90-95%</td>
<td>GC/ECD</td>
<td>14</td>
</tr>
<tr>
<td>0.15 ± 0.02</td>
<td>1.35 ± 0.17</td>
<td>0.91 ± 0.05</td>
<td>1.94 ± 0.19</td>
<td>83%</td>
<td>GC/ECD</td>
<td>15</td>
</tr>
<tr>
<td>1.6 ± 0.22</td>
<td>2.11 ± 0.26</td>
<td>79%</td>
<td>GC/ECD</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.43 ± 0.32</td>
<td>30%</td>
<td>GC/MS</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.69 ± 0.60</td>
<td>91-97%</td>
<td>HPLC</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.34 ± 0.21</td>
<td>93-97%</td>
<td>GC/ECD</td>
<td>Present data</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent mg/24 h ± SE, except for data of references 2 and 3, which represent mg/g creatinine.
Table 2. Effect of Dexamethasone on Urinary MHPG

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Free Basal</th>
<th>Dexamethasone Basal</th>
<th>Sulfate Basal</th>
<th>Glucuronide Basal</th>
<th>Total Basal</th>
<th>Dexamethasone Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.067</td>
<td>0.099</td>
<td>0.91</td>
<td>0.59</td>
<td>0.80</td>
<td>0.34</td>
</tr>
<tr>
<td>2</td>
<td>0.058</td>
<td>0.037</td>
<td>1.32</td>
<td>0.72</td>
<td>1.22</td>
<td>0.64</td>
</tr>
<tr>
<td>3</td>
<td>0.16</td>
<td>0.10</td>
<td>0.97</td>
<td>0.86</td>
<td>0.96</td>
<td>0.53</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>0.095 ±</td>
<td>0.079 ±</td>
<td>1.07 ±</td>
<td>0.72 ±</td>
<td>0.99 ±</td>
<td>0.51* ±</td>
</tr>
</tbody>
</table>

* Dexamethasone sulfate, 0.5 mg every 6 h, orally, eight doses. Urinary MHPG was measured during the second 24 h. Significant difference from basal excretion at p < 0.05 is denoted by *.

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References

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Plasma Glucose Concentrations of Whole Blood, as Determined with a Multilayer-Film Analytical Element
Akiyuki Ohkubo,¹ Sachiko Kamel,¹ Manabu Yamanaka,¹ Fuminori Aral,² Masao Kitajima,² and Asaji Kondo²

A new type of multilayer film analytical element—consisting of a spreading layer, a blocking layer, an enzymic reagent layer, and a transparent layer—has been developed for blood glucose determination. After a spot of whole blood is dropped on the film, the plasma glucose concentration is determined in 6 min without further manipulations. Precise measurement of sample volume spotted on the film is not necessary for good results, nor is knowledge of the hematocrit in the range 10–46%. The minimum concentration of glucose detectable by this method is 100 mg/L. The coefficients of variation, within-run and between-run, were all <2.5% for 800–4000 mg/L glucose concentrations. Macromolecules and hydrophobic substances in blood did not interfere. This method seems suitable for emergency use, especially.

Additional Keyphrases: urgent determinations · multilayer film analysis · reflection densitometry · enzymic methods

It may be helpful for a physician to know the results of blood chemical analysis of his patient right on the spot, when he examines his patient. Prerequisite to this is a simple analytical

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