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Automation and Analytical Techniques

# Fully automated assay for total homocysteine, cysteine, cysteinylglycine, glutathione, cysteamine, and 2-mercaptopropionylglycine in plasma and urine

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**We describe a 6-min HPLC method to measure the total concentrations of the most important thiols in plasma and urine–cysteine, homocysteine, cysteinylglycine, and glutathione–as well as the concentrations in plasma and urine, respectively, of cysteamine and 2-mercaptopropionylglycine, two compounds used to treat disorders of cysteine metabolism. Precolumn derivatization with bromobimane and reversed-phase HPLC were performed automatically by a sample processor. Throughput was up to 100 samples in 24 h. The within-run CV ranged from 0.9% to 3.4% and the between-run CV ranged from 1.5% to 6.1%. Analytical recovery was 97–107%, with little difference between plasma and urine samples. The detection limit was ~50 nmol/L for all the analytes studied. Thiol concentrations were determined in the plasma of 206 healthy donors and in the urine of 318 healthy donors distributed for age and sex. Mean values of plasma cysteine and homocysteine were significantly lower in infants (ages, <1 y) compared with other age groups ($P$ <0.005). In adults, mean plasma homocysteine values were higher in males than in females (9.2 vs 6.7 μmol/L, $P$ <0.0001) and in the 6- to 10-year-old group ($P$ <0.05). Mean values for glutathione and cysteinylglycine were not sex- and age-dependent. In urine, both cysteine and homocysteine showed a wide range of variation.**

The measurement of thiol and disulfide concentrations in human plasma is often useful. Most plasma thiols are metabolically related. Homocysteine, a sulfur-containing amino acid, may either be catabolized to cysteine or remethylated to methionine *(1)*. In addition, cysteine and γ-glutamylcysteine are precursors to glutathione. Cysteinylglycine is derived from the breakdown of glutathione *(2)*.

Disorders of cysteine metabolism include cystinosis, an autosomal recessive disease produced by a defect in lysosomal transport *(3)*, and cystinuria, a common heritable disorder of amino acid transport *(4)*. Cysteamine (β-mercaptoethylamine) depletes cystine from cystinotic cells and is used for treatment of children with nephropathic cystinosis *(5)*. In cystinuria, cystine excretion is reduced by use of sulfhydryl-containing compounds such as 2-mercaptopropionylglycine (MPG)[3] *(6)*.

Hyperhomocysteinemia is found in patients with inborn errors of metabolism that impair the activity of cystathionine synthase *(7)* or the folate and vitamin B12-dependent remethylation process *(8)*. Hyperhomocysteinemia is also associated to the risk of atherosclerosis and premature cardiovascular disease *(9)*.

The measurement of reduced (GSH) and oxidized glutathione in biological samples is essential for evaluation of the redox and detoxification status of cells and tissues in relation to the protective role of glutathione against oxidative and free-radical-mediated cell injury *(10)*.

The concentration of the main plasma thiols in biological fluids can be determined by semiquantitative chemical tests *(11)* or by amino acid analysis. The latter is a

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[3] Nonstandard abbreviations: MPG, 2-mercaptopropionylglycine; GSH, reduced glutathione; and DTT, dithiothreitol.

sensitive but time-consuming quantitative method. Recently, a number of other methods to measure thiols in serum, plasma, and urine have been described *(12–18)*. In this paper, we report a rapid, fully automated HPLC method for total homocysteine, cysteine, cysteinylglycine, and glutathione in plasma and urine and for drug monitoring of cysteamine in plasma and 2-mercaptopropionylglycine in urine. We used this method to estimate reference values for cysteine, homocysteine, cysteinylglycine, and glutathione in plasma and for cysteine and homocysteine in urine in pediatric subjects and adults.

## Materials and Methods

### CHEMICALS

L-Homocystine, L-cystine, cysteinylglycine (reduced form), glutathione (reduced form), cysteamine, 2-mercaptopropionylglycine, *N*-ethylmorpholine, and dithiothreitol (DTT) were obtained from Fluka Chemie; $NaBH_4$, EDTA, 1-octanol, hydrochloric acid, formic acid, acetic acid, acetonitrile, bromobimane, ammonium nitrate, ammonium formate, and NaOH were obtained from Sigma Chemical; dimethyl sulfoxide was obtained from LABSCAN. The column (Hypersil ODS; 150 × 4.6 mm, 3-μm particle size) and the guard column (Hypersil ODS; 10 × 4 mm, 5-μm particle size) were obtained from SGE.

### INSTRUMENTATION

The HPLC system, with sample processor and solvent delivery system, was a Hewlett–Packard Model 1090 M Aminoquant series II; the fluorescence detector was a Hewlett–Packard Model 1046 A operating at an excitation wavelength of 390 nm and an emission wavelength of 478 nm; the data obtained were analyzed with the HP-chemstation program for Windows 3.1 (Hewlett–Packard).

### SAMPLE COLLECTION

Blood was collected into a Vacutainer Tube (Becton Dickinson) containing EDTA. Plasma was obtained immediately by the centrifugation of the blood at 2000*g* for 5 min. Urine was collected into a sterile tube (Becton Dickinson). Plasma and urine were stored at −20 °C until analysis.

### DERIVATIZATION

The derivatization procedure was performed essentially as described by Fiskerstrand et al. *(15)*. The HP-chemstation program controlled several parameters, including the various timing steps necessary for system operation, such as the steps in the derivatization and injection cycle. The autosampler collected 10 μL of 4 mol/L $NaBH_4$ (dissolved in a solution of 333 mL/L dimethyl sulfoxide and 66 mmol/L NaOH), 5 μL of a solution containing 2 mmol/L EDTA and 2 mmol/L DTT, 5 μL of 1-octanol, and 5 μL of 2 mol/L HCl. The mixture was placed in a derivatization vial containing 10 μL of plasma or urine. After incubation for 1 min at room temperature, the autosampler added 25 μL of *N*-ethylmorpholine buffer (2 mol/L, pH 8.0) and 10 μL of 25 mmol/L bromobimane (in 1:1 acetonitrile/$H_2O$, by volume) to the derivatization vial. After incubation for 1 min, the autosampler injected 20 μL of this mixture into the column.

### CHROMATOGRAPHY

The derivatized sample was injected into a 150 × 4.6 mm Hypersil-ODS column equilibrated with 30 mmol/L ammonium nitrate and 40 mmol/L ammonium formate buffer, pH 3.6 (buffer A). The thiols were eluted from the column with a 6-min gradient of acetonitrile (buffer B) (0–4 min, 0–30% buffer B; 4–5 min, 30–100% buffer B; 5–6 min, 100% buffer B) at a flow rate of 1.5 mL/min. The column equilibration time was 5 min, and the column was run at ambient temperature. The retention time for each analyte was calculated using external calibrators at three different concentrations.

### RECOVERY AND PRECISION

Known concentrations of cysteine, cysteinylglycine, glutathione, homocysteine, cysteamine, and 2-mercaptopropionylglycine were added to plasma or urine samples. The concentrations in biological samples with added calibrators were determined in 5 replicates, and analytical recoveries were calculated. The intraassay precision was obtained by analyzing 10 replicates of the biological samples in the same day. The interassay precision was determined by analyzing the same biological samples on 10 different days over 1 month.

### LINEARITY AND LIMIT OF DETECTION

Calibration curves for each analyte (83–333 μmol/L cysteine, 41.7–167 μmol/L cysteinylglycine, 5–83 μmol/L glutathione, 5–83 μmol/L homocysteine, 20–83 μmol/L cysteamine, and 20–83 μmol/L 2-mercaptopropionylglycine) were prepared in duplicate by diluting the stock solutions with 0.1 mol/L HCl containing 100 μmol/L DTT. The linearity of the assays was also studied in the following ranges: 1.5–300 μmol/L cysteine, 0.625–100 μmol/L cysteinylglycine, 0.625–100 μmol/L glutathione, 0.625–100 μmol/L homocysteine, 0.625–100 μmol/L cysteamine, and 0.625–100 μmol/L 2-mercaptopropionylglycine. The limit of detection for calibrator samples, defined as the concentration that produces a signal-to-noise ratio >5, was about 50 nmol/L for all analytes

### STATISTICAL ANALYSIS

The gaussian distribution of the analyte concentrations was tested with use of appropriate tests *(19)*. For statistical analysis, homocysteine values were logarithmically transformed. Differences between analyte concentrations in defined subgroups were determined with the two-tailed Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

## Results

### Optimization of the automated derivatization procedures

We have slightly modified the method used by Fiskerstrand et al. *(15)* to adapt its use to our automated apparatus. Specifically, we increased the final concentrations of $NaBH_4$ (0.57 mol/L instead of 0.19 mol/L) and of bromobimane (3.5 mmol/L instead of 0.8 mmol/L). This did not alter the results of the chromatographic analysis. Moreover, we did not add glacial acetic acid to terminate the reaction because there was no delay between the derivatization with bromobimane and the injection into the column. We found that a reduction of incubation times in our conditions did not alter the results of the analysis.

### Chromatography

The chromatogram of the thiol components of a standard solution is shown in Fig. 1. At pH 3.6, eluted peaks were distinctly separated. Unidentified peaks appeared but did not interfere with the peaks of interest. Cysteine, cysteinylglycine, glutathione, homocysteine, cysteamine, and MPG were eluted in this order with the following retention times (min): 3.7, 3.9, 4.0, 4.1, 4.3, and 5.1. Chromatograms of plasma samples from a healthy individual and a subject with hyperhomocysteinemia are shown in Fig. 2. Chromatograms of urine samples from a healthy subject and from a subject with cystinuria treated with MPG are shown in Fig. 3.

### Precision and recovery studies

Intraassay and interassay precision are shown in Table 1. Within-run CVs for repeatability in plasma measurements were 3.4% for cysteine, 1.9% for cysteinylglycine, 2.8% for glutathione, 2.4% for homocysteine, and 2.8% for cysteamine. For urine samples, intraassay CVs were 2.1% for cysteine and 3.3% for homocysteine. Day-to-day CVs (n = 10) were 4.0% for cysteine, 3.8% for cysteinylglycine, 6.1% for glutathione, 4.9% for homocysteine, and 4.5% for cysteamine in plasma samples. For urine, day-to-day CVs were 3.9% for cysteine and 3.7% for homocysteine. Mean recoveries were 97–107% (Table 2).

### Linearity range and limit of detection

A linear relation was obtained between peak area and thiol concentrations in the ranges studied in an aqueous matrix. Correlation coefficients were >0.99 for all analytes. The equations for the regression line (n = 3) were: $y = 1.66781x + 7.30547$ for cysteine, $y = 0.665378x + 1.06422$ for cysteinylglycine, $y = 0.504754x + 0.263365$ for glutathione, $y = 1.80977x + 0.65961$ for homocysteine, $y = 2.16152x + 5.28814$ for cysteamine, and $y = 2.30103x + 4.94106$ for MPG (where $y$ is the peak area and $x$ is the concentration of the analyte). The lowest concentrations of the linearity studies were all above the limit of detection of 50 nmol/L. No substantial matrix effect was observed when linearity studies were performed on plasma and urine samples diluted up to 1:32 with water containing 100 μmol/L DTT (data not shown).

### Plasma and urine values in pediatric subjects and in adults

As reported in Table 3, total plasma homocysteine, glutathione, cysteinylglycine, and cysteine were determined in 206 healthy donors who attended the outpatient clinic of our hospital; subjects were distributed by age and sex. Urine concentrations of cysteine and homocysteine from 318 healthy donors, also distributed by age and sex, are shown in Table 4. Table 3 shows that plasma cysteine and homocysteine concentrations were markedly lower in infants (ages <1 y) compared with other age groups. No significant differences in plasma cysteine concentrations were seen between males and females in all age groups ($P > 0.05$). Plasma homocysteine concentrations were significantly higher in men ($P < 0.0001$) and in males in the 6–10 y age group ($P < 0.05$), whereas in the age range of 3–6 y, females showed higher plasma homocysteine values. Plasma glutathione concentrations did not show substantial changes according to age and sex (except for higher concentrations in males in the 10–15 y age group).

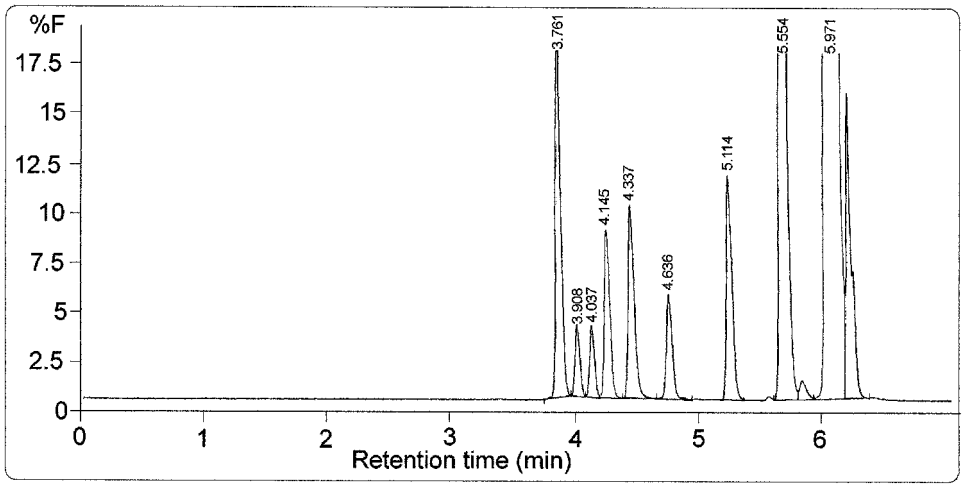


Fig. 1. Chromatograms obtained for calibration solutions containing 100 μmol/L cysteine (3.761 min), 25 μmol/L cysteinylglycine (3.908 min), 25 μmol/L glutathione (4.037 min), 50 μmol/L homocysteine (4.145 min), 50 μmol/L cysteamine (4.337 min), and 50 μmol/L MPG (5.114 min).

Peaks eluted at 4.6, 5.5, and 5.9 min are unidentified compounds caused by bromobimane fluorescence.

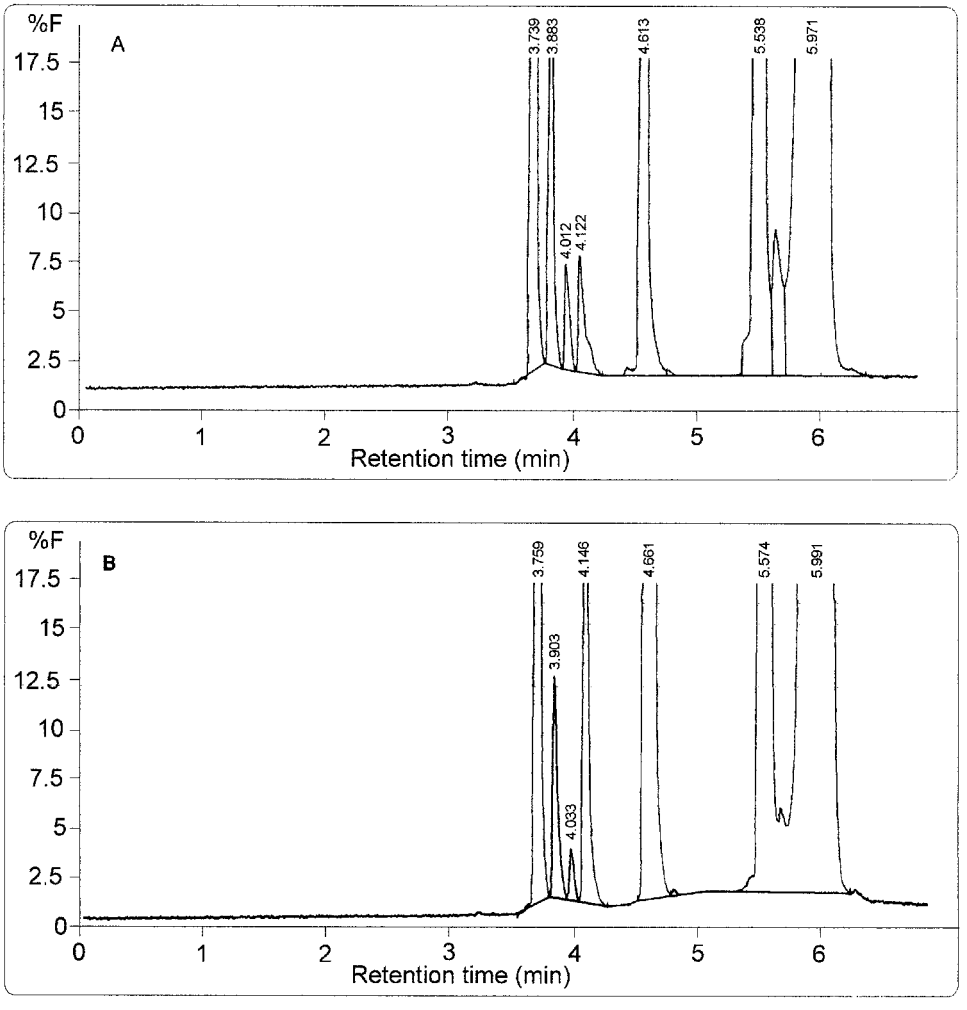


Fig. 2. Chromatograms obtained for plasma samples of a healthy subject (*A*) and a patient with hyperhomocystinemia (*B*).

Cysteine eluted at 3.739 min; cysteinylglycine eluted at 3.883 min; glutathione eluted at 4.012 min; and homocysteine eluted at 4.122 min. Peaks that eluted at 4.6, 5.5, and 5.9 min are unidentified compounds caused by bromobimane fluorescence.

In urine samples, both cysteine and homocysteine are expressed as μmol/mmol creatinine. These values showed a wide range of variation; in particular, females exhibited a substantial increase in urine thiol concentrations between 1 and 3 years of age, whereas, in women, urine homocysteine was lower compared with men and with female infants.

## Discussion

In 1993 Fiskerstrand et al. *(15)* described a fully automated column switching HPLC method for the determination of homocysteine and other thiols in plasma and urine, which was amenable to routine laboratory application because of its simplicity and reproducibility.

We have developed a slight modification of the abovementioned method to achieve the following aims: (*a*) further reduction of analysis times; (*b*) analysis of a wider spectrum of thiol compounds (including drugs) in one run; and (*c*) improvement of peak resolution.

Our method allows the unattended analysis of 100 samples in 24 h. This was obtained by shortening the incubation times in the derivatization procedure (total time of 2 min compared with the 6 min described by Fiskerstrand et al.), which did not interfere with the precision of the chromatographic analysis and the recovery values.

As shown in Fig. 1, the peaks attributed to homocysteine, cysteine, cysteinylglycine, and glutathione are distinctly separated and have good resolution. Under our conditions, the glutathione peak (retention time, 4.037 min) is homogeneous and elutes after cysteinylglycine (retention time, 3.908 min) and before homocysteine (retention time, 4.145 min), which differs from retention times in other reports *(15, 18)*. Two major unidentified peaks appear in chromatograms, but they are distant from our peaks of interest and do not interfere with qualitative and quantitative analysis. The limits of detection of the different thiols (50 nmol/L) were not different from those reported by other authors *(13, 15)*.

Plasma redox thiol status is determined by the measurement of the concentrations of the main detectable components *(20)*. Homocysteine and other aminothiols are present in plasma in separate fractions (protein-bound, free oxidized, and free reduced) *(16)*. In clinical settings, however, most studies measure the total concentration of aminothiols, which is not influenced by the

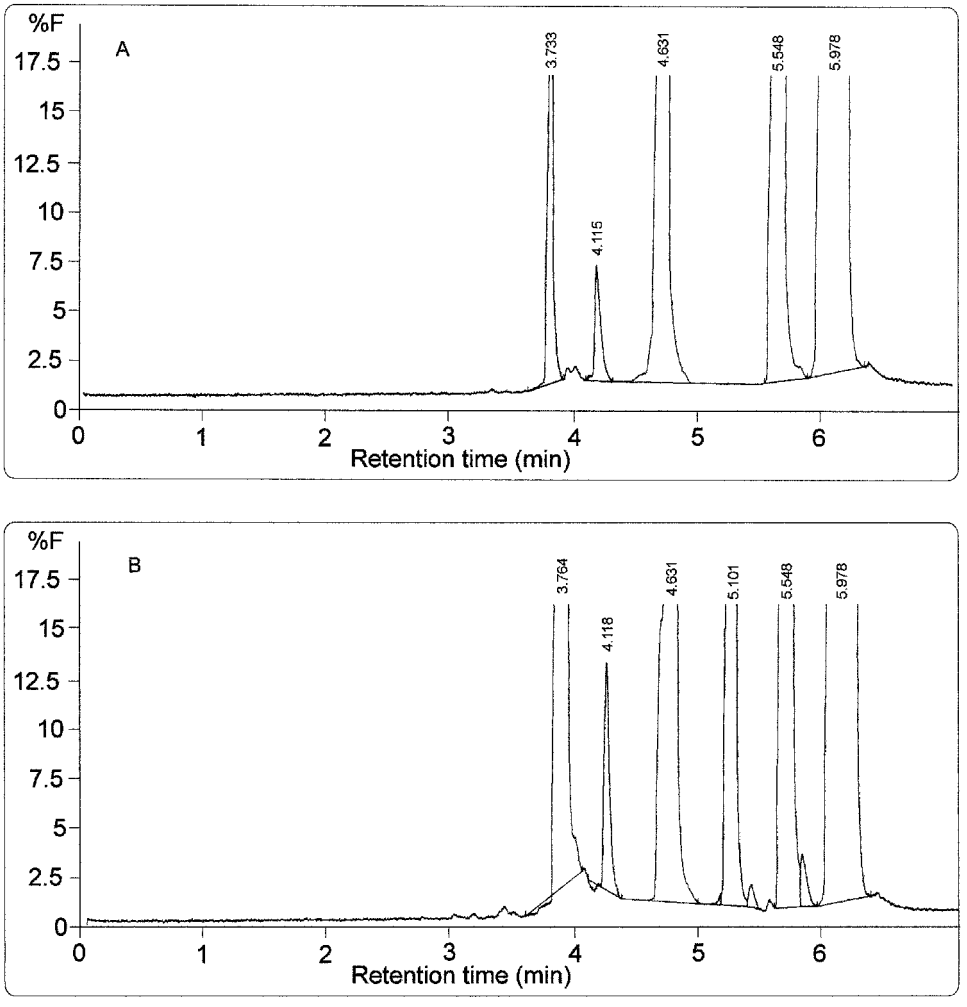


Fig. 3. Chromatograms of urine samples of a healthy subject (*A*) and a patient with cystinuria treated with MPG (*B*).

Cysteine eluted at 3.764 min; homocysteine eluted at 4.118 min; and MPG eluted at 5.101 min. Peaks that eluted at 4.6, 5.5, and 5.9 min are unidentified compounds caused by bromobimane fluorescence.

oxidation and redistribution of the different species as a consequence of sampling procedures. Plasma thiol redox status may be altered by hyperhomocystinemia or increased oxidative stress, which probably affect protein or enzyme functions *(20)*. A moderate increase in plasma homocysteine concentrations is considered an important

**Table 1. Precision of the assay.**

| | Intraassay (n = 10) | | | Interassay (n = 10) | | |
|---|---|---|---|---|---|---|
| | Mean, μmol/L | SD, μmol/L | CV (%) | Mean, μmol/L | SD, μmol/L | CV (%) |
| Plasma | | | | | | |
| Cysteine | 252.3 | 8.70 | 3.4 | 254.2 | 10.2 | 4.0 |
| Cysteinylglycine | 61.7 | 1.15 | 1.9 | 60.9 | 2.30 | 3.8 |
| Glutathione | 11.3 | 0.32 | 2.8 | 11.5 | 0.71 | 6.1 |
| Homocysteine | 12.4 | 0.30 | 2.4 | 12.6 | 0.62 | 4.9 |
| Cysteamine[a] | 3.2 | 0.09 | 2.8 | 3.3 | 0.15 | 4.5 |
| MPG[a] | 83.5 | 1.51 | 1.8 | 84.3 | 1.75 | 2.1 |
| Urine | | | | | | |
| Cysteine | 397.5 | 8.30 | 2.1 | 401.0 | 15.90 | 3.9 |
| Cysteinylglycine[a] | 166.0 | 1.50 | 0.9 | 164.3 | 2.50 | 1.5 |
| Glutathione[a] | 125.4 | 1.27 | 1.0 | 127.2 | 1.90 | 1.5 |
| Homocysteine | 29.2 | 0.97 | 3.3 | 30.1 | 1.12 | 3.7 |
| Cysteamine[a] | 83.2 | 1.17 | 1.4 | 81.9 | 1.35 | 1.6 |
| MPG[a] | 82.9 | 1.15 | 1.4 | 83.1 | 1.40 | 1.7 |

[a] These analytes are not normally detectable. For analytical purposes, we added a fixed amount of each analyte in the sample.

**Table 2. Recovery of the assay.**

| | μmol/L | | | |
|---|---|---|---|---|
| | Sample | Added | Measured[a] | Mean % recovered |
| Plasma | | | | |
| Cysteine | 103 | 333 | 432.8 (1.15) | 99.3 |
| Cysteinylglycine | 68 | 166 | 233.0 (1.12) | 99.6 |
| Glutathione | 12 | 125 | 138.0 (1.58) | 99.3 |
| Homocysteine | 6.8 | 83 | 96.0 (0.79) | 107.0 |
| Cysteamine[b] | 0 | 83 | 81.0 (0.79) | 97.6 |
| MPG[b] | 0 | 83 | 86.1 (0.53) | 104.0 |
| Urine | | | | |
| Cysteine | 181 | 333 | 520.2 (0.84) | 101.0 |
| Cysteinylglycine | 14.7 | 166 | 185.2 (1.0) | 102.0 |
| Glutathione[c] | 0 | 125 | 122.0 (0.79) | 97.0 |
| Homocysteine | 10 | 83 | 95.0 (1.0) | 102.0 |
| Cysteamine[d] | 0 | 83 | 82.5 (1.1) | 99.4 |
| MPG[d] | 0 | 83 | 82.0 (0.79) | 98.0 |

[a] Mean of five replicate values; SD are indicated in parentheses.
[b] Compound not physiologically present in plasma.
[c] Compound not detectable in urine.
[d] Compound not physiologically present in urine.

and independent cardiovascular risk factor *(9)* and may be a useful indicator of vitamin deficiency, especially for folate and cobalamin *(8)*. Tonstad et al. have demonstrated that total plasma homocysteine in young children is associated with premature cardiovascular death in male relatives, usually grandfathers *(21)*. Nevertheless, the importance of moderate hyperhomocysteinemia in children needs to be explored.

In our method, total homocysteine, cysteine, cysteinylglycine, and glutathione were determined in plasma obtained from healthy donors (infants, children, and adults). Substantially higher concentrations of homocysteine were found in plasma from men. This finding is consistent with most other studies, especially if considering that in our study the women were all premenopausal *(17)*. We found no substantial sex-related differences in cysteine concentrations in adults. Infants showed markedly lower plasma concentrations of homocysteine and cysteine in both sex groups in comparison with other age groups. Values in infants ranged from 55% to 70% of the corresponding values in adults. We analyzed values obtained on children by stratifying them into four age groups (1–3 y, 3–6 y, 6–10 y, and 10–15 y); this enabled us to show that sex-related differences in plasma homocysteine concentrations begin to appear after the sixth year of age. In our sample, females between the ages of 3 and 6 years showed the highest absolute values of plasma homocysteine in the female group, and these values were substantially higher than in males of the same age. Sex-related differences in homocysteine concentrations may be mediated by hormones, but the role of estrogen as a modulator of plasma homocysteine has not yet been clarified *(22)*. Our findings seem to confirm that homocysteine concentrations in

**Table 3. Plasma values of cysteine, homocysteine, cysteinylglycine, and glutathione in infants, children, and adults.**

| | Males[a] | | | | | Females[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age, y | CYS[b] | HCY | GSH | CYS-GLY | n | CYS | HCY | GSH | CYS-GLY | n |
| 0–1 | 131 (40)[c] | 5.0 (1.6)[c] | 12.5 (5.2) | 51.7 (1.7) | 10 | 120 (30)[c] | 4.7 (1.2)[c] | 10.0 (7.0) | 51.8 (4.9) | 15 |
| 1–3 | 209 (54) | 10.0 (2.6) | 12.1 (8.9) | 51.1 (7.2) | 15 | 206 (49) | 9.3 (2.3) | 13.2 (6.8) | 63.0 (3.6) | 16 |
| 3–6 | 192 (40) | 8.0 (2.0) | 11.9 (6.9) | 63.4 (7.4) | 11 | 226 (47) | 10.0 (1.3)[d] | 12.7 (6.3) | 56.0 (7.0) | 17 |
| 6–10 | 210 (40) | 10.4 (2.3) | 10.9 (4.5) | 57.2 (7.7) | 13 | 178 (57) | 8.4 (3.0)[e] | 12.3 (4.2) | 54.7 (13.2) | 20 |
| 10–15 | 197 (56) | 9.1 (2.2) | 12.2 (3.1) | 54.0 (18.8) | 23 | 205 (7) | 7.7 (3.0) | 10.2 (3.1)[f] | 57.5 (5.9) | 22 |
| Adults | 212 (23) | 9.2 (2.0) | 12.8 (4.6) | 65.7 (4.6) | 20 | 206 (14) | 6.7 (0.5)[g] | 12.8 (3.6) | 53.2 (7.7) | 24 |

[a] Mean values are expressed in μmol/L; SD are indicated in parentheses.
[b] CYS, cysteine; HCY, homocysteine; GSH, glutathione; CYS-GLY, cysteinylglycine.
[c] Means are significantly different between infants and each other age group at $P < 0.005$.
[d-g] Means are significantly different between sex groups: [d] $P = 0.003$; [e] $P = 0.05$; [f] $P = 0.03$; and [g] $P = 0.0001$.

**Table 4. Urine values of cysteine and homocysteine in infants, children and adults.**

| | Males[a] | | | Females[a] | | |
|---|---|---|---|---|---|---|
| Age, y | Cysteine | Homocysteine | n | Cysteine | Homocysteine | n |
| 0–1 | 32 (10) | 2.1 (1.65) | 28 | 33.76 (9.3) | 1.97 (0.81)[b] | 24 |
| 1–3 | 32.2 (11) | 1.9 (0.69) | 21 | 49 (13.9)[c] | 2.9 (0.75)[c] | 25 |
| 3–6 | 36.3 (12) | 1.9 (0.75) | 38 | 39.7 (14.3) | 1.8 (0.89) | 32 |
| 6–10 | 28 (9.1) | 1.5 (0.69) | 35 | 29.7 (8.3) | 1.4 (0.28) | 28 |
| 10–15 | 34.4 (11.2) | 1.78 (0.73) | 22 | 29.4 (9.3) | 1.86 (2.02) | 21 |
| Adults | 33.4 (15.5) | 1.95 (1.47) | 20 | 28.3 (7.0) | 1.26 (0.38)[d] | 24 |

[a] Mean values are expressed in μmol/mmol creatinine; SD are indicated in parentheses.
[b] Means are significantly different between infants and age groups 1–3, 6–10, and adults, $P < 0.005$.
[c,d] Means are significantly different between sex groups: [c] $P = 0.0001$ and [d] $P = 0.03$.

females decrease with the onset of puberty, that is, in association with rises in estrogen secretion by the ovary.

Urine concentrations of homocysteine and cysteine (expressed as μmol/mmol urine creatinine) did not change substantially throughout the different age groups in males, whereas in females, we observed a particular trend with maximal values in females 1–3 years old and a decrease towards lowest concentrations in adults. Mean homocysteine values in urine from healthy adults were 1.26 and 1.95 μmol/mmol creatinine in females and males, respectively; these results are in good agreement with those reported by other authors who used different methods, such as radioenzymic or capillary gas chromatography–mass spectrometry determinations *(23, 24)*.

A point to be stressed is the importance of evaluating plasma GSH concentrations in addition to homocysteine and cysteine. GSH is the major intracellular nonprotein thiol compound, with concentrations in the millimolar range, whereas plasma concentrations are in the micromolar range *(10)*. Previous studies have demonstrated that blood GSH concentrations serve as an indicator of health status and disease risk, especially in the elderly *(10)*. Mean plasma glutathione concentrations in our sample of adults (12.8 μmol/L) were high in comparison with earlier studies. Paroni et al. *(25)* found a mean glutathione concentration of 7.87 μmol/L in adult healthy donors by using a HPLC method with *o*-phthalaldehyde precolumn derivatization; Svardal et al. *(26)* reported mean values of 5.7 μmol/L, using an assay very similar to that described in our study. Such differences could be due in part to preanalytical factors (i.e., variation in specimen collection and handling). Moreover, in our assay we used higher concentrations of $NaBH_4$, bromobimane, and dimethyl sulfoxide, and we did not add acidic compound as the final step of derivatization; all these variables could affect the analysis. Mean plasma glutathione concentrations measured in males and females in each age group were not statistically different ($P > 0.05$) except for the 10–15 y group ($P < 0.03$). Regarding aging, we observed no substantial variation in plasma GSH concentrations among infants, children, and adults. This is in contrast to data reported by Michelet et al. *(27)*, who observed the lowest plasma GSH concentrations in children and no variation with aging in adults.

Mean plasma cysteinylglycine values were higher in our study compared with earlier reports *(16)*. Such differences could be accounted for by preanalytical and/or analytical factors, as for glutathione.

Sulfhydryl-containing compounds are commonly used as drugs in the treatment of inborn errors of metabolism and in pediatric settings. The use of methods to monitor their concentrations in biological fluids may be important. We devised the HPLC method described above to set up the quantitative analysis of two such drugs.

MPG is a synthetic compound that acts as a potent free radical scavenger *(28)*. It has been shown to protect against ischemic/reperfusion-mediated injury and could also be effective against radiation-induced damages, even at low doses *(28)*. MPG reduces cystine excretion and stone formation and has been proposed as an alternative to treatment with penicillamine in cystinuria, a heritable disorder of amino acid transport with an estimated overall incidence of 1 in 7000 newborns *(4)*. Because of the relatively high frequency of side effects to MPG, some of which are dose-related, drug monitoring in urine samples during treatment is advisable *(4)*.

Cystinosis is a rare lysosomal disorder resulting from the defective carrier-mediated transport of cystine *(29)*. Cysteamine is an aminothiol that can deplete the cystine content of damaged cells *(3)*. In children affected by cystinosis, long-term treatment with cysteamine lowers leukocyte and parenchymal cystine concentrations, improving growth and preserving renal function *(29)*. Severe adverse reactions to oral cysteamine use have been described in subjects in which abrupt incremental dosing of the drug was started *(30)*. This suggests that monitoring the concentrations of cysteamine in biological fluids over the course of therapy is warranted.

In conclusion, this HPLC assay for plasma and urine thiols is sensitive and precise and provides a high sample throughput. The assay is useful for several clinical settings, considering the increasing importance of analytes such as homocysteine and glutathione. The assay is especially advisable for pediatric settings, in which it allows the study of the redox thiol status to be coupled with the monitoring of potentially harmful drugs.

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