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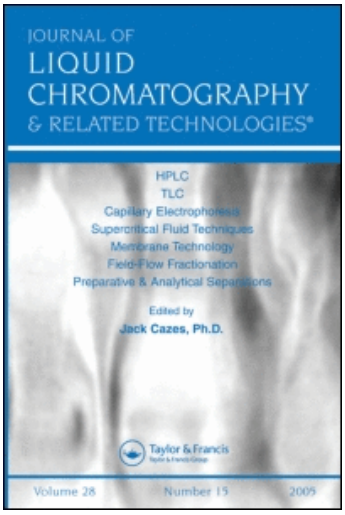


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
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# DETERMINATION OF TOTAL GLUTATHIONE IN CELL LYSATES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH *O*-PHTHALALDEHYDE PRECOLUMN DERIVATIZATION IN THE PRESENCE OF TRIS(2-CARBOXYETHYL)-PHOSPHINE

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Online publication date: 15 November 2000

**To cite this Article** Sack, Ragna , Willi, Annemarie and Hunziker, Peter E.(2000) 'DETERMINATION OF TOTAL GLUTATHIONE IN CELL LYSATES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH *O*-PHTHALALDEHYDE PRECOLUMN DERIVATIZATION IN THE PRESENCE OF TRIS(2-CARBOXYETHYL)-PHOSPHINE', Journal of Liquid Chromatography & Related Technologies, 23: 19, 2947 — 2962
**To link to this Article: DOI:** 10.1081/JLC-100101835
**URL:** http://dx.doi.org/10.1081/JLC-100101835

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J. LIQ. CHROM. & REL. TECHNOL., 23(19), 2947–2962 (2000)

# DETERMINATION OF TOTAL GLUTATHIONE IN CELL LYSATES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH *O*-PHTHALALDEHYDE PRECOLUMN DERIVATIZATION IN THE PRESENCE OF TRIS(2-CARBOXYETHYL)-PHOSPHINE

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## ABSTRACT

A very sensitive HPLC method for the determination of total glutathione (GSH) and γ-glutamyl-cysteine (γ-Glu-Cys) content in cell lysates is presented. It is based on a precolumn derivatization with *o*-phthalaldehyde (OPA) using tris(2-carboxyethyl)-phosphine (TCEP) as a reducing agent. Separation of the peptide derivatives is carried out by reversed-phase chromatography on a C18 column, followed by spectrofluorimetric detection. The fluorescence response of GSH and γ-Glu-Cys derivatives is linear over a range of 0.05 to 100 μM and 1 to 25 μM, respectively, with good precision. The detection limits approach 26 fmol and 60 fmol on column, respectively. In contrast the detection limit for GSH in the presence of DTT, which is often used as the reducing agent, is 250 fmol GSH on column. The results for the GSH concentration of cell lysates are in agreement with a radiotracer method based on metabolic labeling of GSH with [$^{35}$S]-Cys.



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## INTRODUCTION

Glutathione (γ-glutamyl-cysteinyl-glycine, GSH) is a ubiquitous tripeptide occurring in all organisms (reviewed).[1] Its intracellular concentration is in the millimolar range and usually over 99% is present in the reduced form.[2] Intermediates of the GSH biosynthesis such as cysteine, γ-glutamyl-cysteine (γ-Glu-Cys) or cysteinyl-glycine (Cys-Gly) also occur in the cell but at much lower concentrations.[3] Besides its central function in controlling the intracellular redox potential, GSH is involved in the interception of free radicals and reactive peroxides, in the detoxification of cells from high concentrations of heavy metals or alkylating agents, and it also functions as cosubstrate in biotransformation reactions and as enzyme cofactor.

Mirroring the increasing interest in the role of GSH, a number of methods has been reported for its quantification in various biological samples based on enzymatic methods,[4,5] flow cytometry,[6] capillary electrophoresis,[7-11] and HPLC.[12-18] Recently a review focusing on the use of HPLC has been published.[19]

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For the derivatization of primary amino groups with OPA thiol containing compounds are required to form a highly fluorescent isoindole. GSH, containing both a primary amino and a thiol moiety, reacts with OPA at high pH yielding this product without separate addition of a mercaptan.[20] For the quantification of total GSH a reducing agent is needed as GSH is readily oxidized at pH values above 7.[21] Usually 1,4-dithio-DL-threitol (DTT) or β-mercaptoethanol are used as reducing agents.[12,13,15,16,22] However, the presence of such thiol containing compounds beside GSH results not only in the expected GSH-OPA-derivative but also in additional byproducts. In the case of DTT GSH-DTT- and GSH-DTT-OPA-products must be expected which, however, have not yet been described. Tris(2-carboxyethyl)-phosphine (TCEP) is a stable reducing agent acting over a wide pH range,[23,24] which up to now has not been used for the GSH quantification with OPA derivatization although, it is increasingly used in the reduction of disulfide bonds in proteins. We now report an HPLC method allowing the quantification of total GSH and other low molecular weight thiols by precolumn derivatization with OPA in the presence of TCEP as reducing agent. Results obtained by this new HPLC method are compared with those derived from the radiotracer method developed for the investigations on the enhancement of the synthesis of the thiol-rich metallothioneins during cell proliferation.[25]

## EXPERIMENTAL

### Chemicals

Sodium acetate trihydrate, acetonitrile, acetic acid, methanol, *o*-phthalaldehyde (OPA), and 1,4-dithio-DL-threitol (DTT) were purchased from Fluka.

Borate buffer (0.4 N, pH 10.4) was obtained from Hewlett-Packard. Tris(2-carboxyethyl)-phosphine hydrochloride (TCEP) was obtained from Pierce. Tris (Trizma base), sulfosalicylic acid (SSA), glutathione (GSH), glutathione disulfide (GSSG), cysteine (Cys), cysteinyl-glycine (Cys-Gly), and γ-glutamyl-cysteine (γ-Glu-Cys) were from Sigma. Dulbecco's modified Eagle's medium (DMEM), glutamine, gentamycin, amphotericin B, foetal calf serum, and trypsin solution were purchased from Life Technologies, cell culture dishes from Nunc. Scintillant mix (Optiphase HiSafe II) was a product of Wallace. [$^{35}$S]-Cys (specific activity >600 Ci/mmol) was obtained from Du Pont (NEN). All chemicals were of analytical grade. The water used was obtained from an ultra pure-water system (Milli-Q Plus, Millipore).

OPA solution (10 mg/mL) was prepared in 10% methanol (v/v) and was stable at -18°C for at least six months. GSH and γ-Glu-Cys calibration solutions (0.1 mM) were prepared in water. These stock solutions were stable at 4°C for at least one month. The effective GSH, GSSG, and γ-Glu-Cys concentrations were determined by amino acid analysis after gas phase hydrolysis for 6 h at 110°C with 6 N HCl under argon.

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## Cell Culture

Human Chang liver cells (Flow Laboratories) were cultured in DMEM cell culture medium, supplemented with 2 mM glutamine, 50 mg/L gentamicin, 2.5 mg/L amphotericin B, and 10% (v/v) foetal calf serum. Cells were cultured at 37°C, 100% humidity, and 8% $CO_2$. Numbers of cells and cell diameters were determined with a Coulter Counter ZM provided with a Coulter Channelyzer 256 (Coulter Electronics). The average cell volume of 2 pL was calculated from the diameter assuming a globular cell shape.

## Sample Preparation and Quantification of GSH and Cys Based on [$^{35}$S]-Cys Incorporation

Cells were cultured in the presence of [$^{35}$S]-Cys for 72h to achieve isotopic equilibrium prior to seeding for experiments (specific activity 0.93 Ci/mol, corresponding to 0.39 μCi/mL culture medium). Cells grown in the presence of [$^{35}$S]-Cys were harvested and suspended in 25 mM Tris/HCl pH 7.5 at $5.5 \times 10^6$ cells/mL and homogenized by ultrasonification with 25 bursts of 1 sec duration at 10-20 W (Sonifier cell disruptor B15, Branson). The insoluble fraction was removed by ultracentrifugation (100000 x *g* for 60 min). The sample was further purified by ultrafiltration (Ultrafree-CL, exclusion size 30 kDa, Millipore). 0.5 mL of the ultrafiltrate was separated by size exclusion chromatography (SEC) on Superdex Peptide HR 10/30 column (10 mm (i.d.) x 300 mm, Pharmacia) in 25 mM Tris/HCl pH 8.5 with a flow rate of 1 mL/min. Fractions were collected every 30 sec, mixed with 5 mL Scintillant mix, and analyzed by

β-counting (Model LS 1701, Beckmann). The concentrations of total GSH as the sum of GSH and GSSG and cys as the sum of cysteine and cystine were calculated from the specific activity of [$^{35}$S]-Cys.

### Sample Preparation and Quantification of GSH Based on RP-HPLC

Cells were harvested in medium and subsequently dissolved in a freshly prepared solution of 2% SSA (w/v) at $10^6$ cells/mL. Routinely, the samples were mixed thoroughly and subjected to ultracentrifugation (100000 x *g* for 60 min). To confirm complete disintegration of the cells, a part of the sample was subjected to ultrasonification (see above) with 25 bursts of 1 sec duration at 10-20 W prior to ultracentrifugation. Figure 1 presents an overview of different sample preparation procedures applied.

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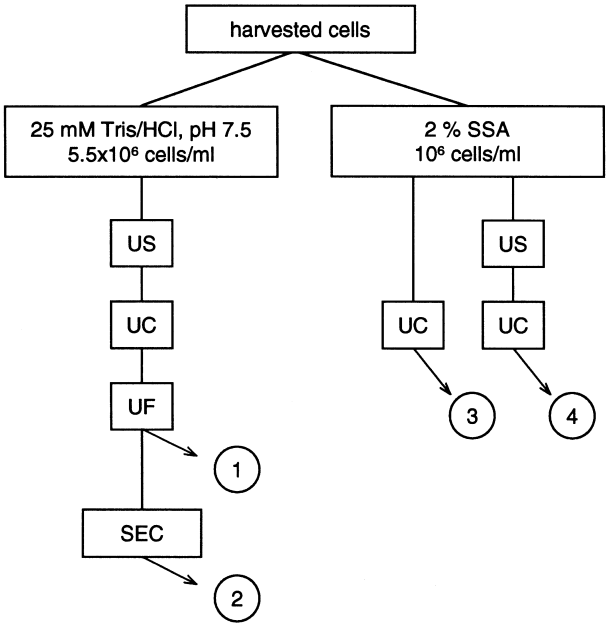


**Figure 1**. Overview of sample preparation for both methods for GSH determination. Cells grown in the presence of [$^{35}$S]-Cys were harvested and prepared for analysis as described in the experimental section. The numbers indicate sample aliquots taken at different sample preparation steps. 1. Aliquot taken from partially purified cell extract; 2. aliquot taken from a single fraction eluting from SEC; 3. cell homogenate directly subjected to ultracentrifugation; 4. cell homogenate further prepared by ultrasonification. US: ultrasonification, UC: ultracentrifugation, UF: ultrafiltration.

### RP-HPLC

Prior to chromatographic analysis, the samples were mixed with equal volumes of TCEP (5.0 mM) or DTT (5.0 mM). The chromatographic system consisted of a HP 1090M liquid chromatograph with a stand-alone HP1046A programmable fluorescence detector and an HPLC ChemStation/Pascal Series (Hewlett-Packard). The samples were derivatized with OPA in an autosampler using an injector program as follows: 5 µL borate buffer, 2 µL GSH-standard or sample-solution, 0.5 µL OPA solution, 2 minutes reaction time before injection. Separation was performed on an ODS Hypersil column (2.1 mm (i.d.) x 200 mm, 5 µm particle size, AminoQuant, Hewlett-Packard) protected by a guard column (ODS Hypersil, 2.1 mm (i.d.) x 20 mm, 5 µm particle size, Hewlett-Packard) at 30°C. Elution was carried out with a gradient formed between mobile phase A (30 mM sodium acetate, adjusted to pH 6.0 with acetic acid) and mobile phase B (92.3% methanol/7.7% acetonitrile, v/v). The following elution program was used: from 4% to 14.9% B in 15 min at a flow rate of 0.45 mL/min. The OPA-derivatives were monitored at an excitation and emission wavelength of 230 nm and 445 nm, respectively.

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The system was calibrated daily using three GSH solutions of different concentration (5, 25, 50 µM) each containing TCEP (2.5 mM). The within-day precision was evaluated for GSH (25 µM), GSSG (44.5 µM), and GSH + GSSG (25 µM + 44.5 µM) in the presence of TCEP (2.5 mM) by at least triplicate analysis.

For comparative studies solutions of 0.05, 0.1, 0.5, 1.0, 5.0, and 10 µM GSH were analyzed. Each concentration in the presence 2.5 mM of either TCEP or DTT was measured ten times.

## RESULTS AND DISCUSSION

### Influence of TCEP and DTT

To obtain the maximum OPA-GSH-derivative yield, the optimum concentration of the reducing agent TCEP was evaluated by examining various TCEP concentrations in the reaction mixture. For this purpose, solutions of GSH, GSSG, and a mixture of both were prepared and treated with increasing amounts of TCEP. Table 1 shows that for the efficient reduction and subsequent derivatization of 25 to 70 pmol GSH equivalents of GSH and GSSG the optimum amount of TCEP was 5 nmol on column, corresponding to a 72 to 200-fold molar excess of TCEP in the sample mixture. Although, with 20 nmol TCEP on column for the reduction of 70 pmol GSH equivalents comparable results were obtained, the GSH recovery was significantly reduced at higher amounts of TCEP. These data indicate that the derivatization is generally disturbed at TCEP amounts above 5 nmol on column unless the molar excess is not

**Table 1**

**Recovery of Total GSH in Dependence on the TCEP Concentration**

| Amount Injected[a] | | | Amount Measured[a] | |
|---|---|---|---|---|
| GSH (pmol) | GSSG (pmol) | TCEP (nmol) | GSH (equ.) (pmol) | Recovery (%)[b] |
| 25 | | 5 | 27.7 ± 0.3 | 110.9 ± 1.5 |
| | 22.25 | 5 | 44.3 ± 0.7 | 99.5 ± 2.0 |
| 25 | 22.25 | 5 | 70.3 ± 0.5 | 101.1 ± 0.9 |
| 25 | | 20 | 22.8 ± 0.4 | 91.2 ± 1.9 |
| | 22.25 | 20 | 41.2 ± 0.5 | 92.6 ± 1.3 |
| 25 | 22.25 | 20 | 68.1 ± 1.4 | 98.1 ± 2.4 |
| 25 | | 100 | 12.5 ± 1.4 | 50.0 ± 6.8 |
| | 22.25 | 100 | 34.3 ± 8.3 | 77.0 ± 23.0 |
| 25 | 22.25 | 100 | 8.1 ± 4.5 | 11.6 ± 8.0 |

[a] Total GSH values are expressed as GSH equivalents of GSH and GSSG (GSH (equ.)). [b] For calculation of the recovery the amount injected is set to 100. Data are means ± standard deviation, n = 3.

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higher than about 300-fold. However, at the TCEP amount of 5 nmol on column as applied for routine analysis, the molar excess of TCEP over GSH equivalents has no influence on the reaction as indicated by the good correlation coefficient of the calibration (see below).

A reduced recovery, and consequently, an increased detection limit of the OPA-GSH-derivative would be presumed if DTT is used for the reduction of GSH and GSSG. DTT possesses two thiol groups and is best suited to act not only as reducing agent but also as the mercaptan in the derivatization reaction. To investigate this effect, GSH solutions of five different concentrations were analyzed in the presence of either 2.5 mM TCEP or 2.5 mM DTT (Figure 2). As expected, with DTT as reducing agent, a decreased yield of OPA-GSH-derivative was confirmed which is in contrast to some published data.[12,13,22] Not more than about 80% of the expected GSH equivalents could be determined in the presence of DTT. This correlates with the recovery of 73% found by Parmentier et al.[16] It signifies that in the presence of DTT 0.2 pmol GSH on column are not detectable. The detection limit with DTT as reducing agent approaches 0.25 pmol GSH on column which is about 10 times higher than that in the presence of TCEP.

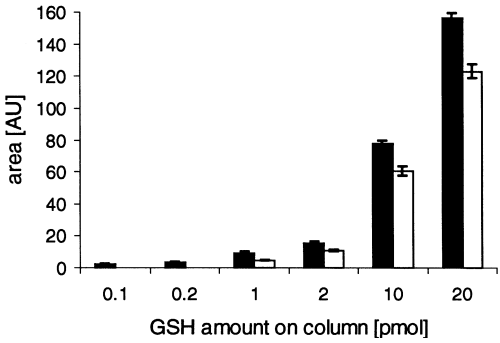


**Figure 2**. Comparison of the GSH-OPA recoveries at different GSH amounts in the presence of 2.5 mM TCEP (black bars) and 2.5 mM DTT (white bars). Error bars indicate the standard deviation (n = 10).

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## Detection Limit, Linearity, and Reproducibility

Linearity was given over a range of 0.1 to 20 pmol on column (correlation coefficient = 1.000). The regression line showed a slope of 7.38 and an intercept on the y-axis of - 0.2 (n=10). The detection limit was calculated to be 26 fmol GSH on column. The within-day precision was evaluated for GSH, GSSG, and GSH + GSSG solutions. The appropriate coefficients of variation of the peak area were 1.4%, 2.2%, and 1.6%, respectively (n=10).

## Recovery of GSH

The effects of interfering substances on GSH determination were evaluated by standard addition. Known amounts of GSH (5, 25, 50 pmol) were added to a sample partially purified by ultrasonification, ultracentrifugation, and ultrafiltration (sample 1, see Figure 1). As the measured values correlated with the expected amounts of GSH the complex sample matrix did not influence the GSH recovery (Figure 3). No additional signals were detected, indicating that no other fluorescent derivative was formed or present under the given conditions.

Under the chosen chromatographic conditions the OPA-GSH-derivative eluted as a single peak (Figure 4), and even when SSA was used as a deproteinizing agent, GSH could readily be detected and quantified (Figure 4, C, D). However, attention must be paid to the SSA concentration in order to avoid the influence of SSA on GSH detection and quantification. At higher concentrations of SSA the GSH-OPA-derivative is not completely resolved and elutes on

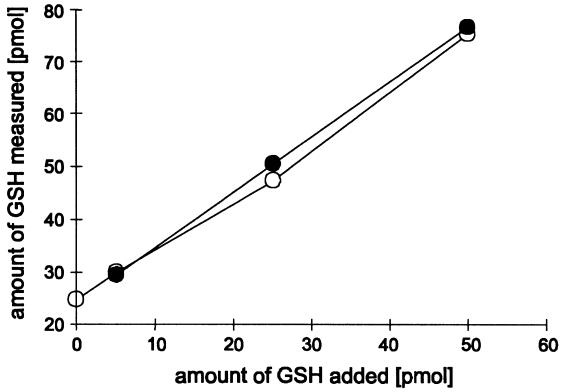


**Figure 3**. Evaluation of the recovery of total GSH in partially purified cell extract of sample 1 (see Figure 1) by addition of different amounts of GSH standard (5, 25 and 50 pmol). The measured GSH concentration (open symbol) is compared with the expected GSH concentration as the sum of GSH concentration in sample 1 plus the amount of added GSH standard (closed symbols).

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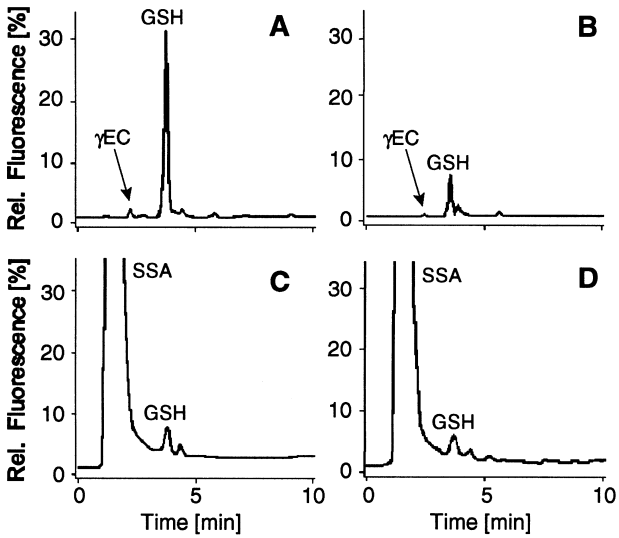


**Figure 4**. Chromatograms of cell extracts at different purification steps with OPA-derivatization in the presence of 2.5 mM TCEP followed by HPLC. 2 µL of the samples specified in Figure 1 were analyzed. Chromatographic conditions are given in the experimental section. Chromatogram A: sample 1, partially purified cell extract; B: sample 2, single fraction after SEC; C: sample 3, cell homogenate directly subjected to ultracentrifugation; D: sample 4, cell homogenate additionally treated by ultrasonification. γ-EC: γ-Glu-Cys, GSH: glutathione, SSA: sulfosalicylic acid.

the tailing edge of the SSA peak. For best results the SSA concentration in the sample preparation solution should not exceed 2% (w/v). Likewise, the SSA solution should be prepared daily, because a significant broadening of the SSA peak is observed when older SSA solutions are used.

### Recovery of Other Low Molecular Weight Thiols

Besides GSH and GSSG, other low molecular weight thiols are present in the cells. Therefore, the HPLC method was tested for the determination of γ-Glu-Cys and Cys-Gly. Although both compounds, like GSH, contain a primary amino and a thiol group, only the γ-Glu-Cys formed a detectable fluorescent derivative. The different reactivity of Cys-Gly may be explained by the different relative positions of the amino and thiol group in these molecules. While in γ-Glu-Cys like in GSH, itself, the two reactive centers are located in two adjacent amino acids, in Cys-Gly and Cys, they are part of the same amino acid. Thus, possibly due to steric reasons the formation of the fluorescent intramolecular isoindole is inhibited. In the presence of 2.5 mM TCEP the calibration curve of γ-Glu-Cys was linear over a range of 1.5 to 50 pmol on column (correlation coefficient = 0.998), corresponding to a concentration of 0.75 to 25 μM in the calibration solution. The detection limit was calculated to be 60 fmol on column, indicating that γ-Glu-Cys may easily be quantified if cell extracts are prepared in Tris-buffer as shown for sample 1 (Figure 1). In contrast, using SSA as deproteinizing reagent (samples 3 and 4, Figure 1), the γ-Glu-Cys peak is covered by the large peak eluting with the solvent front containing SSA (Figure 4, C and D).

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**Table 2**

**Stability of OPA-GSH-Derivatives**

| Reaction Time (min)[a] | Area (AU)[a] | Relative Recovery (%)[b] |
|---|---|---|
| 0 | 309.9 ± 1.2 | (100) |
| 1 | 317.6 ± 1.7 | 102.5 |
| 2 | 306.1 ± 2.0 | 98.8 |
| 5 | 290.0 ± 1.3 | 93.6 |
| 10 | 289.5 ± 7.3 | 93.4 |

[a] 5 μL Borate buffer (0.4 N, pH 10.4), 2 μL GSH (22 μM) containing 2.5 mM TCEP and 0.5 μL OPA (10 mg/mL in 10% methanol (v/v)) were mixed in the autoinjector and injected after distinct time intervals. [b] Data are means ± standard deviation, n = 3).

### Stability of the OPA-Derivatives

It is known that OPA/thiol-derivatives formed with primary amino group containing compounds are relatively unstable depending on the thiol used.[26] Therefore, we tested the stability of the OPA-GSH-derivative by determination of the GSH yield for increasing time intervals (0, 1, 2, 5, 10 min) between preparation of the reaction mixture and injection onto the column (Table 2). The reaction was very fast and was completed after about 1 min. After that time, degradation started and significantly decreased GSH recoveries were found for reaction times longer than 2 minutes. To detect small amounts of GSH and to achieve high reproducibility it is, therefore, important to follow a strict time table with an optimum of a 1 to 2 minutes reaction time.

### Stability of Cell Extract Fractions

Selected cell extract fractions were stored at –20°C over 5 months and then their GSH concentrations were determined again by HPLC (Figure 5). Obviously SSA containing samples were not stable. The differences in the GSH concentration of samples 1 and 2 (see Figure 1) are very small and can be attributed to the experimental error, thus documenting that GSH was stable in Tris-

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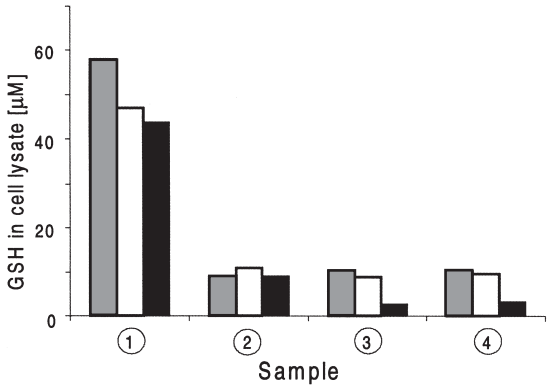


**Figure 5**. Comparison of total GSH concentration in cell lysates at different purification steps determined by the radiotracer method (gray bars), HPLC with OPA-derivatization in the presence of 2.5 mM TCEP (white bars) and HPLC with OPA-derivatization in the presence of 2.5 mM TCEP after a storage period of 5 months (black bars). Sample 1, partially purified cell extract; sample 2, single fraction after SEC; sample 3, cell homogenate directly subjected to ultracentrifugation; sample 4, cell homogenate additionally treated by ultrasonification. The circled numbers correspond to those in Figure 1.

buffer. This allows single sample collection over extended time periods with one final HPLC analysis.

**Comparison of GSH-Concentrations Obtained by the HPLC and Radiotracer Method**

The in-house radiotracer method originally developed for the detection of the thiol-rich metallothioneins,[25] was used for comparison of the GSH values obtained by the HPLC method. In the radiotracer method cells grown to isotopic equilibrium in the presence of [$^{35}$S]-Cys were suspended in Tris-buffer and the lysate was purified over several steps, separated by SEC and analyzed by β-counting. The cellular GSH concentration was then calculated from the sum of all GSH containing fractions eluting from SEC.

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To verify the results obtained by the HPLC method, a single fraction eluting from SEC was analyzed by β-counting (sample 2, Figure 1). In Figure 5, the GSH concentrations obtained by the two methods are summarized for each sample. For sample 2, the values of the GSH concentration correlate well. The small difference of about 1 to 2 μM is attributed to the experimental error of each method which is, however, in this order of magnitude acceptable for biological systems. Thus, for samples purified over several steps according to the procedure for the detection of the radioisotopes the two methods are equivalent.

In the radiotracer method the SEC is necessary to separate GSH from cysteine. With respect to the HPLC method, cysteine with a concentration in the very low millimolar range (1.3 mM)[25] does not interfere with the OPA-derivatization of GSH and the fluorescence detection of the OPA-GSH-derivative. Therefore, an aliquot of the purified cell lysate was submitted to HPLC analysis before SEC (sample 1, Figure 1). The resulting GSH concentration (Figure 5) is about 14% lower than the GSH concentration determined by the radiotracer method as the sum of all GSH containing fractions eluting from SEC (Figure 6). Considering an estimated experimental error of about 5%, the observed difference in the total GSH concentration of sample 1 is not astonishing for two reasons. Firstly, as can be seen in Figure 6, the GSH peak is not fully baseline separated from the cys peak (as the sum of both cysteine and cystine). Secondly, at given conditions γ-Glu-Cys and Cys-Gly coelute with GSH (data not shown). According to other studies, the cellular concentrations of γ-Glu-Cys and Cys-Gly are about 3.5 and 2.0% of the cellular GSH concentration, respectively.[3] The γ-Glu-Cys concentration of samples 1 and 2 measured by the HPLC method accounts for about 4% of the total GSH concentration (Figure 4), which is in good agreement with these studies. By assuming a value of about 2% for Cys-Gly, the value for the GSH concentration in sample 1 determined by the radiotracer method is augmented by at least 6% in comparison to the HPLC method. Thus, the total value deduced from the sum of all GSH containing fractions eluting from SEC (Figure 6) must be at least 11%

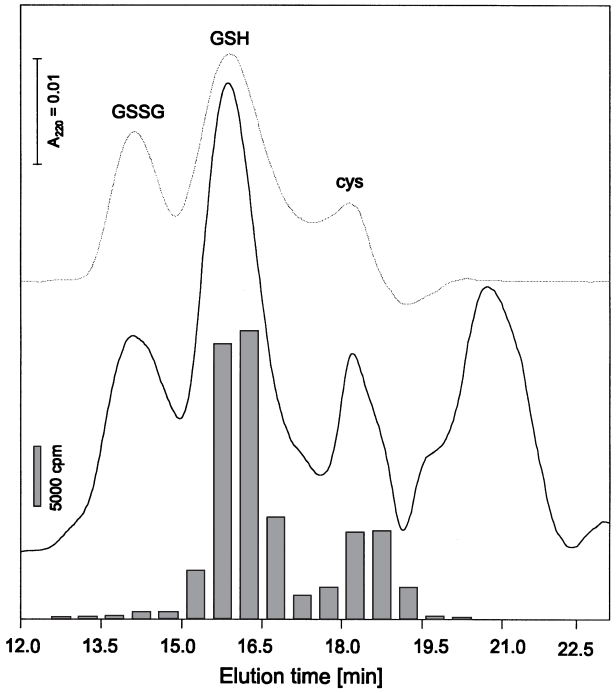


**Figure 6**. Quantification of total GSH (i.e. GSH and GSSG) and cys (i.e. cysteine and cystine) by [$^{35}$S]-Cys incorporation. Chang liver cells grown in the presence of [$^{35}$S]-Cys to isotopic equilibrium were disrupted by ultrasonification. The low molecular solute fraction was collected by ultracentrifugation and ultrafiltration and resolved by SEC (see Experimental). The elution profile was monitored by absorbance at 220 nm (solid line), where the positions of GSH, GSSG and cys were identified with standards (dotted line). Bars represent the corresponding elution profile of [$^{35}$S]-Cys. GSH and cys were quantified based on the specific radioactivity employed in the cell culture.

higher than the actual GSH concentration in sample 1 determined by the HPLC method. In summary, for the determination of GSH by the HPLC method, the SEC separation step can be eliminated and the results are more accurate than those derived from the radiotracer method.

The purification of cell lysates necessary for the radiotracer method is rather time consuming. Therefore, for the HPLC method a completely different purification procedure was tested. Cell lysates were treated with SSA and the precipitates were removed by ultracentrifugation (sample 3, Figure 1). An

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additional ultrasonification step was introduced for sample 4 (Figure 1) to ensure complete cell disruption. In spite of the complex matrix in which GSH was analyzed for both samples, the results derived from the HPLC methods correlate well with those deduced from the cellular GSH concentration determined by the radiotracer method (Figure 5). Additionally, the comparison of the GSH concentrations of sample 3 and 4 determined by the HPLC method indicates that the ultrasonification step is not needed for complete recovery of GSH from the cells. That means that the purification procedure for GSH from cell lysates can be minimized to a single precipitation step with SSA before HPLC analysis.

The cellular concentration of total GSH of 9.1 nmol/$10^6$ cells (as average of the cellular GSH concentrations obtained from sample 1, 3 and 4) determined by the HPLC method correlates well with the result calculated for the radiotracer method (10.6 nmol/$10^6$ cells). With a cell volume of 2 pL the corresponding GSH concentrations of 4.5 and 5.3 mM, respectively, are in good agreement with other studies.[2]

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## CONCLUSION

The reversed-phase HPLC method described in this report allows accurate quantification of total glutathione (GSH) in biological samples. The detection limit for GSH is 20 times lower in the presence of TCEP than in the presence of DTT allowing the detection of GSH traces. GSH can be quantified after a single protein precipitation step with SSA. In parallel the quantification of γ-Glu-Cys is also possible if no SSA is used for deproteinization of the sample. The results are verified by an in-house radiotracer method. Because of the higher sensitivity, the use of TCEP will greatly facilitate investigations of the specific role of GSH in cellular proliferation and other important cellular processes.

## ACKNOWLEDGMENTS

This work was supported by the Swiss Science Foundation Grant No. 3100-040807.94/1 and by the Kanton of Zurich/Switzerland.

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Received May 10, 2000 Author's Revisions July 10, 2000
Accepted June 15, 2000 Manuscript 5316

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