Determination of Thiols in Yeast by HPLC Coupled with LTQ-Orbitrap Mass Spectrometry after Derivatization with *p*-(Hydroxymercuri)benzoate

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A liquid chromatography method with mass spectrometric detection has been developed for the simultaneous determination of six thiols in the sulfur metabolic pathway, including cysteine (Cys), homocysteine (HCys), glutathione (GSH), cysteinyl-glycine (Cys-Gly), γ-glutamyl-cysteine (Glu-Cys), and *S*-adenosyl-homocysteine (AdoHcy). Tris(2-carboxyethyl)phosphine (TCEP) was used as the reducing reagent and *p*-(hydroxymercuri)benzoate (PHMB) as the derivatization reagent. Thiols were extracted from 3 mg of yeast using water in an ultrasonic bath. The absolute detection limits for the compounds studied were in the subpicomole range. It was found that AdoHcy, Cys, GSH, Cys-Gly, Glu-Cys, and very little HCys were present in the selenium-enriched yeast sample studied, and GSH, Glu-Cys, very little AdoHcy, Cys, and Cys-Gly were present in three bakery yeasts.

KEYWORDS: Thiol; derivatization; LTQ-Orbitrap mass spectrometry; yeast

INTRODUCTION

Endogenous low-molecular-weight thiols, such as glutathione (γ-glutamyl-cysteinyl-glycine, GSH), cysteine (Cys), and homocysteine (HCys), and their corresponding disulfides (glutathione disulfide (GSSG), cystine, and homocystine) are critical cellular components. In the sulfur metabolic pathway, Cys and γ-glutamyl-cysteine (Glu-Cys) are endogenous precursors of GSH. The biosynthesis of the tripeptide GSH occurs in two steps, the first of which is the synthesis of Glu-Cys catalyzed by γ-glutamylcysteine synthetase. The addition of a glycine residue is then catalyzed by glutathione synthetase. The degradation of glutathione is catalyzed by γ-glutamyl transpeptidase and results in the formation of cysteinyl-glycine (Cys-Gly) (*1*). HCys is formed from endogenous s-adenosyl-homocysteine (AdoHcy). HCys is an intermediate in the metabolism of methionine, and it can either be catabolized to Cys or remethylated to methionine (*2*, *3*). There is also auto-oxidation of HCys to homocystine (*4*).

Thiols circulate as free thiols, disulfides, −NO and −OH derivatives, and protein-bound complexes. It is widely accepted that thiols participate in the processes of metabolism, antioxidant defense, and drug detoxification, and alterations in the concentrations and the ratio of free thiols and disulfides can be recognized as biomarkers of the metabolism and redox status in biochemical, physiological, pharmacological, and toxicological studies. Therefore, analytical methods for the simultaneous detection and quantification of these thiols in biological samples are of great importance.

The main challenges in the assay of thiols in biological samples are due to their unfavorable physicochemical properties. Thiols are highly polar and water-soluble compounds, and it is usually difficult to separate them from complex biological matrices without derivatization. Most of the thiols are present at low concentrations in biological samples, and a selective and sensitive detection system for the determination of these compounds is often required. Thiols are also labile due to the high reactivity of the free sulfhydryl (−SH) group and convert easily to disulfides via an auto-oxidative process (*5*). Therefore, it is important to stabilize these −SH groups in biological samples immediately after sample collection to prevent conversion of thiol compounds.

The most common step to stabilize the thiols is to derivatize them with a suitable derivatization reagent, which can react instantaneously and quantitatively under suitable conditions. Usually the derivatization complexes are more hydrophobic than thiols; thus, separation of the derivatization complexes from other polar compounds in the biological matrices becomes much easier.

Many derivatization reagents for the stabilization of thiols have been described in the literature. The most widely used ones are *N*-ethylmaleimide and its analogues, but they have some disadvantages. The primary fluorescent adducts of the thiol derivatives are unstable and are converted into the secondary fluorophores at the N−C=O position of maleimide. A prolonged reaction time is required to completely convert the primary fluorophore into the secondary one; otherwise, two peaks for fluorophores are observed in the chromatogram (*6*). The combination of a thiol compound with *N*-ethylmaleimide would form a new chiral center on the *N*-ethylmaleimide side of the thiol

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adduct. If a chiral center already exists in a thiol compound, such as L-cysteine, two pairs of diastereomeric derivatives are generated, and two peaks would show up as a result (*7, 8*).

Ellman's reagent (5,5′-dithiobis(2-nitrobenzoic acid), DTNB) is widely used for the analysis of thiols via the detection of the Ellman anion (ES$^-$) generated in the reaction. However, another Ellman-based species (ESO$_2$$^-$) generated by a side reaction would be an interferant and the side reaction would lead to an overestimation of the thiol concentration (*9*).

Another widely used derivatization reagent is Sanger reagent (2,4-dinitro-1-fluorobenzene, DNFB, DNPF, FDNB), but it has been reported that cyst(e)ine and γ-glutamyl-cyst(e)ine in lichen *Pseudevernia furfuracea* could not be detected after derivatization with Sanger reagent. These thiols were detected after labeling with monobromobimane (mBBr), but this method is complicated and time-consuming (*10*).

Some other derivatization reagents described in the literature, such as 2-chloro-1-methylquinolinium and 2-chloro-1-methylquinolinium tetrafluoroborate (CMQT), were synthesized by the authors themselves in the laboratory (*11, 12*) and are not commercially available.

For this study, an organic mercury probe, *p*-(hydroxymercuri)benzoate (PHMB), was chosen as the derivatization reagent. PHMB has the advantage of only reacting with one sulfhydryl group, while some other mercury reagents such as divalent mercury ions (Hg^{2+}) have the potential to react with two sulfhydryl groups or existing disulfide bonds (*13*). On the basis of this characteristic, PHMB has served as a mass spectrometry tag for counting the number of sulfhydryl groups and disulfide bonds in peptides and proteins (*14*). PHMB has also been applied to the protein quantification, and it improves the detection sensitivity of ovalbumin significantly in comparison to the use of sulfur as a naturally occurring elemental tag (*15*). The PHMB derivatization reaction is very fast. It has been reported that PHMB reacts with −SH groups at room temperature in less than 90 s with high affinity and specificity. Thiol-PHMB complexes maintain the solubility of the noncomplexed peptides (*16*). Thiol-PHMB complexes are stable for the length of the working day if kept at room temperature or 3 months if stored at −20 °C (*17*). Due to the unique isotope envelope of the mercury tag, the actual identification of the thiols is greatly simplified. The mercury in PHMB can also be detected by atomic fluorescence spectrometry (AFS) or inductively coupled plasma mass spectrometry (ICP-MS). A method based on AFS using PHMB labeling for the quantification of thiols in urine was published recently, and it showed good sensitivity (*18*).

Disulfides contain no −SH and cannot be derivatized with PHMB directly; therefore, a reducing reagent is needed to reduce the disulfides to free thiols. Several reducing agents have been described in the literature: sodium borohydride (NaBH$_4$), dithiothreitol (DTT), 2-mercaptoethanol, and phosphine derivatives, such as tris(2-carboxyethyl) phosphine (TCEP) and tri-*n*-butylphosphine (TBP). In this study, TCEP was selected as the reducing reagent on the basis of efficiency, reaction time, water solubility, and stability in both acidic and basic solutions (*19−23*).

After derivatization, high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) was used as the analytical system. Finally, the developed method was applied to the analysis of thiols in yeast.

One author has reported the use of 25% ethanol (v/v) to extract GSH from yeast (*24*), but most of the previous studies focused on extraction of thiols in water using an ultrasonic bath, which requires a long extraction time (at least 3 h) (*25, 26*). The major limitation in using such a long extraction time for thiols is the possibility of changes to the original sample composition due to thiol oxidation or degradation.

The extraction of Se-thiols by accelerated solvent extraction (ASE) has also been reported (*24*), but whether thiols can withstand such a high temperature (100 °C) is doubtful.

An ultrasonic probe (*27*) and glass beads (*28, 29*) have also been used for the disruption of yeast cells.

In previous studies, large amounts of yeast sample, 250−300 mg, were used (*25, 26, 30−32*). In this study, 3 mg of yeast was extracted for 10 min in an ultrasonic bath, and more than 97.4% of thiols in the yeast was extracted. The sample processing procedure was simple and fast, and using such a small amount of sample could be an advantage when studying the metabolomics of yeast. In summary, our extraction method was efficient and practical in terms of sample amount, simplicity, and extraction time.

MATERIALS AND METHODS

Reagents and Solutions. AdoHcy, Cys, HCys, GSH, Cys-Gly, Glu-Cys, cystine, homocystine, GSSG, TCEP, and PHMB (*p*-(hydroxymercuri)benzoic acid, sodium salt) were purchased from Sigma−Aldrich Chemical Co. (Oakville, ON, Canada).

Methanol was HPLC grade and was obtained from EMD Chemicals Inc. (Darmstadt, Germany). Formic acid and ammonium hydroxide were purchased from Anachemia Canada Inc. (Montreal, QC, Canada). High-purity water was generated using a MILLI-Q-ADVANTAGE (A10) system from Millipore Corporation (Saverne, Alsace, France).

Stock solutions of AdoHcy, Cys, HCys, GSH, Cys-Gly, and Glu-Cys (1 mmol/L) were prepared in water, and cystine, homocystine, and GSSG (1 mmol/L) were prepared in 0.1 mol/L hydrochloric acid to improve their solubility. A stock solution of TECP (40 mmol/L) was prepared in water. A stock solution of PHMB (10 mmol/L) was prepared by dissolving the sodium salt in 0.1% NH$_4$OH.

Samples. Selenium-enriched yeast, bread machine yeast, instant yeast, and active dry yeast were obtained locally.

Sample Pretreatment. About 3 mg of yeast was accurately weighed in an Eppendorf tube, 600 μL of water was added, and the sample was vortex-mixed for 1 min. After extraction for 10 min in an ultrasonic bath, the sample was centrifuged (12 000 rpm, 5 min), and 250 μL of the supernatant was transferred to an Eppendorf tube.

Derivatization Procedure. A 50 μL portion of TECP (5 mmol/L) was added to the tube, and the sample was vortex-mixed for 10 s. After incubation at room temperature for 15 min, 50 μL of PHMB (3.5 mmol/L) was added. Thirty minutes later, the sample was centrifuged (12 000 rpm, 5 min), and 10 μL of the supernatant was analyzed by LC-MS.

High-Performance Liquid Chromatography and Tandem Mass spectrometry. Samples were analyzed using an 1100 Series HPLC system from Agilent Technologies, Inc. (Palo Alto, CA). The HPLC consisted of an autosampler, a quaternary pump, a Zorbax SB-C8 (2.1 × 100 mm, 3.5 μm) column, and a guard column (Zorbax SB-C8, 2.1 × 12.5 mm, 5 μm). The mobile phase flow rate of 0.2 mL/min was achieved with an elution gradient composed of solvent A (0.1% formic acid in water) and of solvent B (0.1% formic acid in methanol). The gradient was as follows: 10% solvent B for 1 min; 9 min linear increase up to 30% solvent B; 10 min linear increase up to 90% solvent B; 7 min linear decrease to 10% solvent B; 10% B hold for 8 min. The injection volume was 10 μL, and the total analysis time was 35 min.

Detection was performed on a LTQ-Orbitrap mass spectrometer manufactured by Thermo Fisher Scientific Inc. (Bremen, Germany) which was equipped with an electrospray ion source. Samples were analyzed using both the Iontrap and Orbitrap mass spectrometers. The Orbitrap was operated at resolution settings of 15 000, 30 000, 60 000, and 100 000. Full-scan spectra over the *m/z* range 335−685 were acquired in the positive ion mode. The voltage on the electrospray needle was set to 3 kV. The capillary temperature was set to 300 °C. The sheath and auxiliary gases were both nitrogen at flow rates of 20 and 5 arbitrary units, respectively. The tube lens voltage was set to 95 V, and the capillary voltage was set to 39 V. Ions with *m/z* values of 385, 444, 458, 630, 501, and 573, which correspond to the protonated molecular ions of AdoHcy and PHMB-derivatized Cys
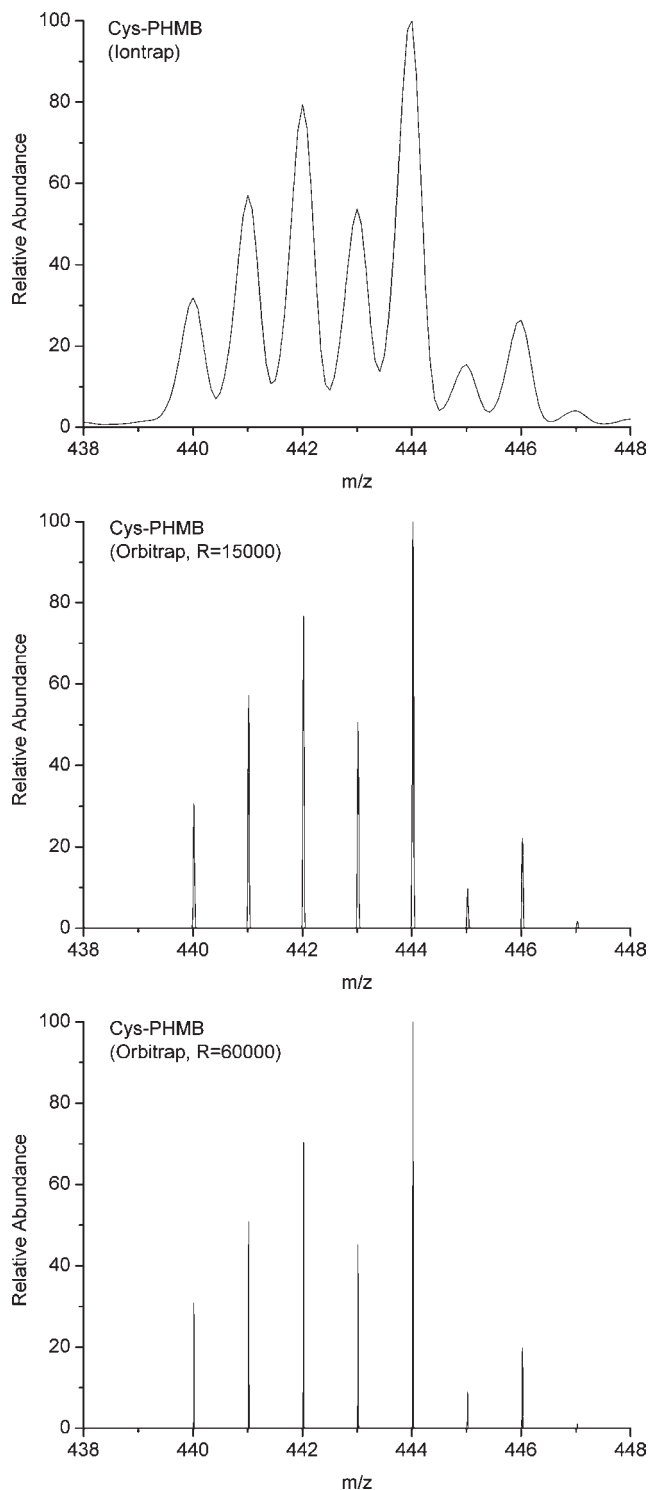
Figure 1. Mass spectra of Cys-PHMB using the Iontrap and Orbitrap at resolutions of 15 000 and 60 000.

(Cys-PHMB), HCys (HCys-PHMB), GSH (GSH-PHMB), Cys-Gly (Cys-Gly-PHMB), and Glu-Cys (Glu-Cys-PHMB) were extracted from the full-scan spectra. Data were processed using Xcalibur software (Thermo Fisher Scientific, Inc., Bremen, Germany), version 2.0.7.

Figures of Merit of the Analytical Protocol. *LOD.* The LOD was defined as the analyte concentration calculated by using 3 times the standard deviation of the intensity of the noise observed when a blank solution is injected, divided by the slope of the calibration curve. LODs for both the standard solutions and yeast samples were determined.

Linear Range. Two series of calibration samples each covering a range of 0.5−15 μmol/L for each thiol were prepared by mixing known amounts
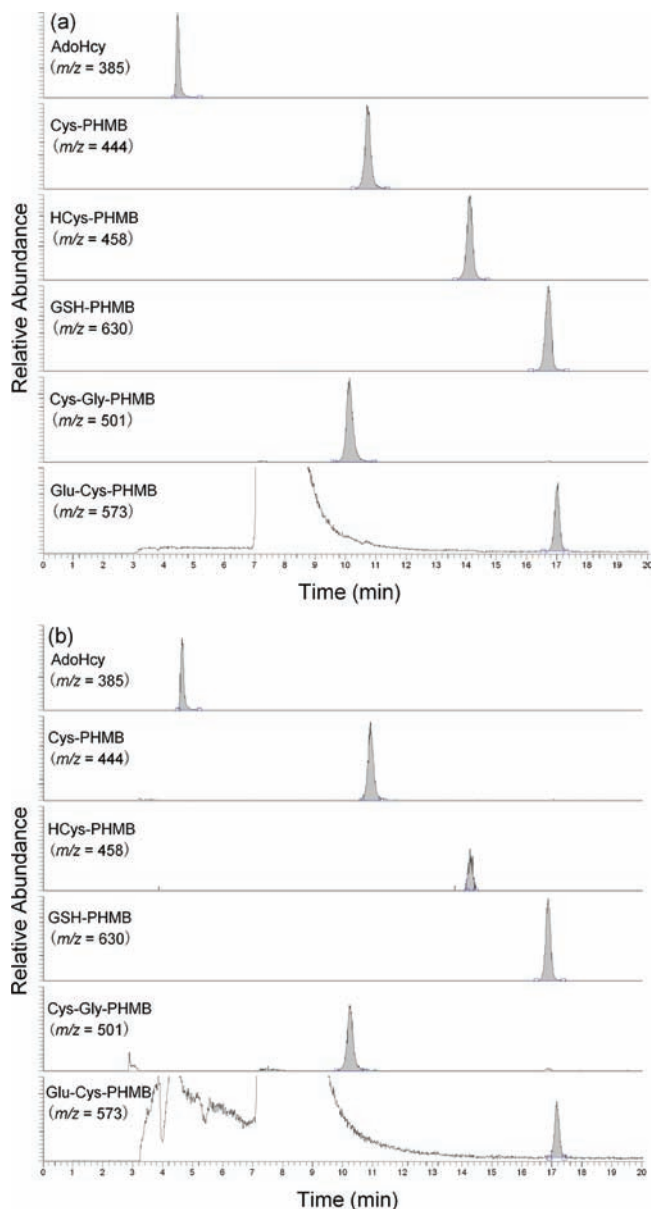

Figure 2. Representative chromatograms of (a) a mixture of free thiol standards including AdoHcy, Cys (as Cys-PHMB), HCys (as HCys-PHMB), GSH (as GSH-PHMB), Cys-Gly (as Cys-Gly-PHMB), and Glu-Cys (as Glu-Cys-PHMB) at a concentration of 3 μmol/L each and (b) thiols in the selenium-enriched yeast sample. The *m/z* value of the ion being monitored in each channel is indicated in the figure. The peak before Glu-Cys-PHMB at *m/z* 573 is the TCEP-PHMB complex (RT = 7.4 min), which has an *m/z* value very similar to that of Glu-Cys-PHMB (the mass difference is 0.001 Da).

of the thiol standards with water and yeast. Five-point calibration curves were obtained by least-squares linear regression analysis of the thiol peak area ratio vs the thiol concentration in the calibration sample. The slopes of the calibration curves for water and yeast extract were compared to evaluate matrix effects.

Precision and Accuracy. Six replicate yeast samples were spiked with thiols (5 μmol/L) and analyzed to obtain the precision of the method, expressed as %RSD. Accuracy was assessed by determining yeast samples spiked with thiols (5 μmol/L) and expressed as (mean observed concentration)/(spiked concentration) × 100%.

Stability Study. A yeast sample was spiked with thiols (3 μmol/L of each thiol), derivatized, and injected multiple times over 46 h, and the peak areas were compared.

Application to Yeast Samples. The proposed method was applied to the determination of total thiols in selenium-enriched yeast and three bakery

yeast samples. A standard addition method was used for quantification, and thiol standards were added before extraction.

RESULTS AND DISCUSSION

LC/MS Analysis. Figure 1 shows the mass spectra of Cys-PHMB at various mass resolutions. Because of the unique isotope envelope of the mercury tag, the actual identification of the thiols was greatly simplified. **Figure 2** shows representative LC/MS chromatograms of a mixture of standards (**Figure 2a**) and a selenium-enriched yeast sample (**Figure 2b**).

Study of the Concentration of TCEP and PHMB. Derivatization of disulfides (1 mL of a mixture of cystine, homocystine, GSSG) with a concentration of 5 μmol/L each) was performed by mixing the solutions with 50 μL of TCEP with concentrations of 12, 20, and 28 mmol/L as the reducing reagent. The mixtures were incubated for 10 min and then mixed with 100 μL of PHMB (1−8 mmol/L) and allowed to stand for 30 min. The best combination of peak shapes and derivatization efficiency was obtained with 20 mmol/L of TCEP and 7 mmol/L of PHMB, which was chosen for future experiments.

Study of the Reduction Time. Derivatization of disulfides (1 mL of a mixture of cystine, homocystine, and GSSG, at a concentration of 5 μmol/L each) was performed by mixing the solutions with 50 μL of TCEP (20 mmol/L) as the reducing reagent for varying incubation times and then adding 100 μL of PHMB (7 mmol/L) and letting the mixture stand for 30 min.

As can be seen in **Figure 3**, reduction times between 1 and 20 min do not influence the response of the mercury complex formation significantly. A 15 min incubation time for the reduction phase was used for subsequent derivatizations. These results are consistent with the literature (*21*), where it has been reported that TCEP
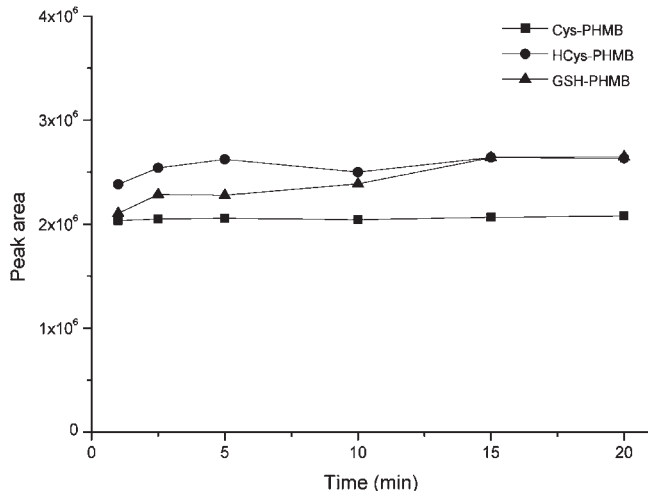

Figure 3. Effect of incubation time when disulfides (1 mL of a mixture of cystine, homocystine, and GSSG, at a concentration of 5 μmol/L each) were reduced with 50 μL of TCEP (20 mmol/L) and then derivatized with 100 μL of PHMB (7 mmol/L).

reduction of HCys is complete within 1 min or so at room temperature.

Optimization of the Extraction of Thiols from Yeast. The overall goal for the extraction method development was to be able to process a large number of samples simultaneously with a typical sample size in the low milligram range.

Initially, water with or without the addition of methanol was used as the extraction solvent for the extraction of thiols from yeast. It was found that the addition of methanol for the extraction of the highly polar thiol compounds provided no additional benefit.

An ultrasonic probe, ultrasonic bath, and glass beads were tested as disruption methods. No significant differences were observed among these approaches. The ultrasonic bath was chosen for further use, since smaller amounts of sample could be treated and more samples could be extracted at the same time, in comparison to the ultrasonic probe method.

Different sonication times (0−90 min, with ice in the ultrasonic bath) were studied, and no significant change with time was observed. Ten minutes was then chosen as the sonication time for subsequent extractions.

Extraction efficiency was also determined. Three replicate yeast samples were extracted three times in an ultrasonic bath for 10 min with water as the extracting solvent. Thiols were determined in each of the three extracts of each sample. The results showed that more than 97.4% of the thiols in yeast was extracted in the first step (97.4−100% first step, 0−2.6% second step, 0−0.3% third step).

The sample processing procedure was simple and fast, and the oxidation of thiols could be minimized. Small amounts of yeast sample (3 mg) can be used for each analysis, which will be an advantage when this method is applied to metabolic studies.

Figures of Merit for the Analytical Protocol. The sensitivity of the Iontrap and Orbitrap were compared in terms of LOD. The integration windows for each of the scan methods are shown in **Table 1**, and the LODs of thiols in both standard solutions and yeast samples are shown in **Table 2**. The results show that, for most of the analytes, the Orbitrap provided better LODs than the Iontrap, though the differences are not large. The advantage of high mass resolution became obvious when the complex yeast extracts were analyzed. The Orbitrap, at resolution 30 000, was chosen for use in the rest of the study.

The absolute LODs for the thiols were in the subpicomole range, and they were better than for the LC-UV methods (*11, 12, 33*), most of the MS-based detection methods (*5, 34−39*), and the results via fluorescence measurements (*40, 41*).

The relationship between detector response and thiol concentrations was demonstrated, and the calibration curves were linear up to 15 μmol/L for the compounds studied.

The precision of the method was established by analysis of six replicate yeast samples, and the RSDs were no more than 9%. Extraction yields for six thiols ranged from 95 to 107%.

In order to study the effect of matrices, two different sets of calibration samples were prepared by adding the reduced thiols to

Table 1. Integration Ranges for Each Scan Method

		Orbitrap (*m/z*)			
	Iontrap (*m/z*)	15 000	30 000	60 000	100 000
AdoHcy	384.50−385.50	385.11−385.16	385.12−385.15	385.12−385.14	385.12−385.14
Cys	443.50−444.50	443.99−444.06	444.00−444.05	444.01−444.04	444.01−444.03
HCys	457.50−458.50	458.00−458.07	458.01−458.06	458.02−458.06	458.02−458.05
GSH	629.50−630.50	630.02−630.15	630.04−630.12	630.07−630.11	630.07−630.10
Cys-Gly	500.50−501.50	501.00−501.09	501.02−501.07	501.03−501.06	501.03−501.05
Glu-Cys	572.50−573.50	573.01−573.12	573.04−573.09	573.05−573.09	573.05−573.08

water and to yeast. The slopes of the calibration curves were compared to evaluate the matrix effects. No significant differences between calibration slopes in the water and yeast matrices were observed. It may be concluded that the analysis of thiols in yeast is not influenced significantly by the sample matrix. This finding is consistent with the literature (*19*).

As shown in **Figure 4**, the thiols after derivatization were stable at room temperature over a time period of 46 h. It has been reported in the literature that thiol-PHMB complexes are stable for the length of the working day if kept at room temperature (*17*). **Figure 4** also shows the instrument variation over a 2 day period.

Results of Yeast Analysis. The quantification of thiols in yeast samples was carried out using selenium-enriched yeast and three bakery yeast samples (machine yeast, instant yeast, active dry yeast). Standard addition was used for the quantification of thiols. The results of measured concentrations of thiols in yeast are shown in **Table 3**.

It is known that selenium is a cofactor of glutathione peroxidase (GPX), one of the important antioxidant enzymes, and that *Saccharomyces cerevisiae* has three GPX homologues (*42*).

However, the role of selenium in thiol metabolism in yeast has not been studied. The concentration of total GSH (reduced and oxidized) in bakery yeasts was in the range of 14.63−18.34 μmol/g, and it was consistent with the value reported in the literature (*43*) (about 16.27 μmol/g). Interestingly, we found that the concentration of GSH decreased by 3−4 times and Glu-Cys by about $^1/_3$ in selenium-enriched yeast. Both these peptides are substrates of γ-glutamyl transferase (GGT), an enzyme involved in the transport and metabolism of GSH (*44*). GGT converts Glu-Cys to Cys and GSH to Cys-Gly, and Cys-Gly is converted to Cys and Gly by L-Cys-Gly dipeptidase (*45*). This could explain the increase of Cys-Gly and Cys concentration. AdoHcy and HCys are involved in the *S*-adenosyl-methionine (SAM) cycle, which consists of the reactions that produce, consume, and regenerate SAM. In the first step of this cycle, the SAM-dependent methylases (EC 2.1.1) that use SAM as a substrate produce AdoHcy as a product (*46*). AdoHcy is hydrolyzed to HCys and adenosine by *S*-adenosyl-homocysteine hydrolase (EC 3.3.1.1) and the HCys recycled back to methionine and methionine converted back to SAM, completing the cycle (*47*). The concentration of AdoHcy and HCys increased in selenium-enriched samples: very little HCys was found in the selenium-enriched yeast, and no HCys was found in the bakery yeasts. In the literature (*48*), no HCys could be detected in yeast either, and there was a rapid increase of HCys in response to cadmium treatment. It was reported that the concentration of HCys in yeast was about 20 times less than the concentration of Cys (*49*). The amount of HCys in other

Table 2. LODs of Thiols in Standard Solutions and Yeast Matrix

	Iontrap	Orbitrap			
		15 000	30 000	60 000	100 000
Standard Solution (nmol/L)					
AdoHcy	4	2	2	2	1
Cys	18	12	11	18	8
HCys	48	9	9	10	8
GSH	7	5	5	2	1
Cys-Gly	9	22	15	18	24
Glu-Cys	25	15	26	36	32
Yeast (nmol/g)					
AdoHcy	5	1	1	0.3	0.3
Cys	4	7	3	3	2
Hcys	5	2	2	2	1
GSH	5	3	1	1	1
Cys-Gly	4	4	2	3	3
Glu-Cys	5	2	3	4	7

Table 3. Concentrations of Thiols Extracted from 3 mg Yeast Samples Determined using LC with the Orbitrap Mass Spectrometer at 30 000 Resolution[a]

	concn (μmol/g)			
analyte	selenium-enriched yeast	bread machine yeast	instant yeast	active dry yeast
AdoHcy	0.26	0.01	0.02	n.d.
Cys	0.46	0.07	0.07	0.08
HCys	0.02	n.d.	n.d.	n.d.
GSH	5.20	18.34	17.69	14.63
Cys-Gly	0.61	0.02	0.01	0.05
Glu-Cys	0.17	0.64	0.47	0.52

[a] n.d. = not detected. RSD: 8−13% ($n = 5$).

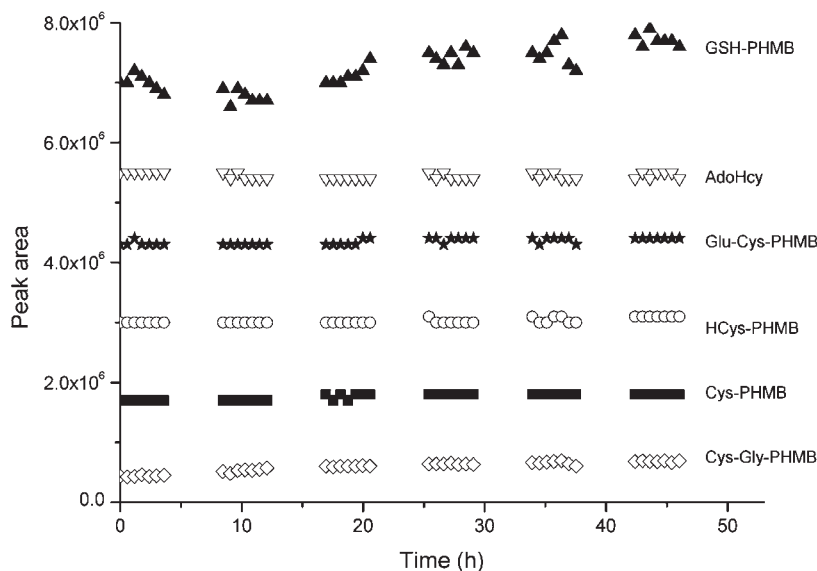**Figure 4.** Stability study of thiols in yeast after spiking at a concentration of 3 μmol/L (replicate data points are offset for better visual representation of the data).

biological samples (such as urine and plasma) was also quite low, compared with Cys (the concentration ratio between Cys and HCys ranging from 14:1 to 41:1) (*5*, *11*, *34*, *38*, *50*). According to this ratio, the concentration of HCys should be 11−33 nmol/g, which would be at or near the LOD (2 nmol/g). It has been reported that about 70% of the homocysteine in human plasma is associated with plasma proteins (*51*). The possibility and degree of protein binding of HCys in yeast remains to be investigated.

These results might suggest an involvement of selenium in thiol metabolism, not limited to its role as an enzyme cofactor or as an S analogue in S-containing amino acids. Thus, the developed methodology could be fundamental to future metabolic studies.

ABBREVIATIONS USED

Cys, cysteine; HCys, homocysteine; GSH, glutathione; Cys-Gly, cysteinyl-glycine; Glu-Cys, γ-glutamyl-cysteine; AdoHcy, *S*-adenosyl-homocysteine; TCEP, tris(2-carboxyethyl)phosphine; PHMB, *p*-(hydroxymercuri)benzoate; GSSG, glutathione disulfide; GPX, glutathione peroxidase; GGT, γ-glutamyl transferase; SAM, *S*-adenosyl-methionine.

SAFETY

PHMB is a toxic chemical, and Material Safety Data Sheets should be consulted.

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