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Jean-Luc Boudenne

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1 **Fast microplate assay for simultaneous determination of thiols and dissolved sulfides in**
2 **wastewater**

3
4 Bruno Coulomb^{1*}, Fabien Robert-Peillard¹, Edwin Palacio², Robert Di Rocco¹, Jean-Luc
5 Boudenne¹

6
7 ¹ Aix Marseille Univ, CNRS, LCE UMR7376, 13331 Marseille, France.

8 ² Laboratory of Environmental Analytical Chemistry. University of the Balearic Islands, E-
9 07122 Palma de Mallorca, Illes Balears, Spain.

10
11 * corresponding author: Tel: +33 4 13551028 – Fax: +33 4 13551060

12 Email address: bruno.coulomb@univ-amu.fr
13

14
15 **Abstract**

16 The present paper reports the development and the application of a microplate assay for the fast
17 and individual determination of thiols and sulfides. The method is based on the reaction of ethyl
18 propiolate with thiols and sulfides, yielding thioacrylates absorbing in the UV range.
19 Thioacrylate generated by derivation of sulfide with EP presents a bathochromic shift of
20 maximal absorbance wavelength of 16 nm compared to thioacrylates formed with thiols. EP
21 concentration, reaction time and derivatization pH were carefully optimized in order to
22 separately quantify thiols and sulfides using a spectral deconvolution procedure in 15 minutes.
23 The detection limits were 0.6 and 2.8 μM (63.7 and 92.4 $\mu\text{g.L}^{-1}$) for mercaptopropionic acid
24 (used as reference thiol compound for calibration) and dissolved hydrogen sulfide respectively.
25 Non-thiol sulfur compounds (methionine, sulfates and sulfites) or other nucleophile compound
26 did not display any interference in the determination of MPA or sulfides. Interference from
27 metals was suppressed with application of a reduction step with TCEP and DTPA. Finally, the
28 method has been successfully applied to the analysis of raw and treated wastewater.

29

1 **Keywords:** thiols; ethyl propiolate; hydrogen sulfide; high-throughput analysis; UV spectral
2 deconvolution.

3

4 **1. Introduction**

5 Malodorous emissions and odor control in collection and treatment of wastewater remain a
6 major concern for operators of wastewater treatment plants (WWTP). Organic and inorganic
7 compounds, directly or indirectly responsible for odors, are emitted by sewage or result from
8 the anaerobic degradation of organic matter containing sulfur or nitrogen. Among these
9 compounds, the two main inorganic odorous compounds are hydrogen sulfide and ammonia
10 [1], but we can also mention thiols, indoles and other inorganic or organic nitrogen and sulfur
11 compounds. Complex mixtures of sulfur compounds at low concentrations (range from $\mu\text{g}\cdot\text{m}^{-3}$
12 to $\text{mg}\cdot\text{m}^{-3}$) form the majority of these malodorous emissions associated with sewage treatment
13 works. Hydrogen sulfide (H_2S), carbon disulfide (CS_2), methyl mercaptan or methanethiol
14 (CH_3SH), dimethyl sulfide [$(\text{CH}_3)_2\text{S}$] and dimethyl disulfide [$(\text{CH}_3)_2\text{S}_2$] were the most
15 frequently identified compounds in influent and primary effluent of a WWTP [2,3].

16 Thiol compounds are often quantified by separation methods like gas chromatography coupled
17 to flame photometric detector [4] or mass spectrometer [3], or like high performance liquid
18 chromatography (HPLC) with pre-column derivatization coupled to fluorescence [5-8], UV
19 detection [9] or mass spectrometry [10]. Several studies have also focused on the global
20 quantification of thiol levels mainly in biological systems by absorbance [11,12], fluorescence
21 measurement [13,14] or enzymatic activity [15]. Many easy-to-use microplate kits, usually
22 based on the use of the Ellman's method or fluorescent maleimide derivatives and calibrated
23 using standard solutions of glutathione and/or cysteine, are commercialized for the
24 measurement of free or total thiols in biological samples. Traditional Ellman's reagent can also
25 be used for inorganic sulfides determination and numerous other analytical techniques reported

1 for hydrogen sulfide determination in natural waters or sewage may be mentioned including
2 spectrophotometry [16,17], fluorimetry [18], HPLC coupled to UV-Vis detector [19],
3 inductively coupled plasma-atomic emission spectroscopy [20] or flow systems [21,22].

4 Many analytical systems exist for hydrogen sulfide analysis in the gas phase. However, it is
5 also important to monitor dissolved sulfides and thiols so as to predict H₂S emissions at
6 facilities such as sewage treatment plants or to control the quantities of chemical inhibitors to
7 add to a wastewater stream. Some authors have developed methodologies based on multi-
8 wavelengths exploitation of UV-Vis spectra for the determination of sulfides in natural waters
9 [23] or dissolved organic sulfide (DOS) in industrial wastewater [24], but these techniques
10 require pH buffering of samples and suffer from relatively high detection limits.

11 Recently, Owen (2008) has described the reaction of thiol compounds with esters of propiolic
12 acid (like ethyl propiolate, EP) under mild conditions to form stable and strongly UV absorbing
13 thioacrylates [25]. Tzanavaris et al. (2010) have adapted this derivatization method as a
14 sequential injection analysis system for the determination of thiols in pharmaceutical samples
15 [26]. Even though these two studies demonstrated good selectivity of this derivation method,
16 our preliminary tests showed that sulfides could also react with ethyl propiolate to form a stable
17 derivative with a red shift of the maximum absorption wavelength of about twenty nanometers
18 as compared to thiols derivatives (Fig. S1).

19 In the present work, we decided to optimize the derivatization properties of ethyl propiolate
20 (EP) to develop a new and simple spectrophotometric high-throughput assay in a 96-well plate
21 for simultaneous determination of thiols and sulfides in wastewater. The assay was validated
22 by comparison with Ellman's and OrthoPhtalAldehyde-AminoEthanol (OPA-AE) methods on
23 real wastewater samples.

24

25 **2. Experimental**

1 2.1. Reagents and solutions

2 All chemicals used in this study were of analytical reagent grade. Ethyl propiolate, sodium
3 sulfide nonahydrate, mercaptopropionic acid, mercaptoacetic acid, dimethyldisulfide,
4 ethanethiol, tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and diethylene triamine
5 pentaacetic acid (DTPA) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier,
6 France), cysteine, glutathion, 2-mercaptoethanol, *o*-phthaldialdehyde and 5,5'-dithiobis(2-
7 nitrobenzoic acid) (DTNB) were from Alfa-Aesar (Schiltigheim, France). EP and other thiol
8 compounds stock standard solutions and working solutions were prepared in ultrapure water
9 (Millipore, resistivity >18 MΩ cm).

10

11 2.2. Instrumentation

12 Microplate absorbance and fluorescence measurements were carried out on a microplate reader
13 (Infinite M200, Tecan France SAS, Lyon, France) controlled by i-control™ software (Tecan)
14 in clear polystyrene 96 flat-bottom well microplates for absorbance or black polystyrene 96 V-
15 bottom wells shape microplates for fluorescence (Fisher Scientific, Illkirch, France).

16

17 2.3. Analysis of thiols and dissolved sulfides

18 A protocol for simultaneous determination of thiols (R-SH) and sulfides (HS⁻) has been
19 developed at the microplate level. In addition, a reduction step by TCEP has enabled the
20 determination of total thiols (R-SH + R-S-S-R' + R-S-Me-S-R', with Me a metal and R and R'
21 a functional group) by measuring variation of signal after and before reduction. To compare the
22 performance of this analytical method, thiols determination was also performed with two other
23 methods traditionally used for this purpose and optimized in microplate format: OPA-AE
24 derivatization method and Ellman's method. The various protocols are described below.

25

1 2.3.1. High throughput assay for reduced and total thiols, and dissolved sulfides determination
2 by EP derivatization

3 The reduction of total thiols was carried out by adding 10 μ l of TCEP 20 mM in a buffer solution
4 (0.2 M borate pH 9.5 and DTPA 2 mM) to 100 μ L of sample or standard. The plate was shaken
5 and after 8 min 50 μ L of EP 50 mM in 0.2 M borate buffer pH 9.5 was added and absorbance
6 spectrum was measured between 230 and 350 nm after 2 min for total thiols determination and
7 15 min for dissolved sulfides determination.

8 The same protocol can be used for reduced thiols determination by directly dispensing 100 μ L
9 of sample into the wells of the microplate, without the reduction step by TCEP. Both protocols
10 are summarized in Scheme 1. Quantification of total or reduced thiols and dissolved sulfides
11 was carried out by spectral deconvolution using ACD/Spectrus Processor software (ACD/Labs
12 2012, Totonto, Canada) and the SIMPLISMA (SIMPLe-to-use Interactive Self-modeling
13 Mixture Analysis) tool, based on a two-stepped regression procedure using the normalized
14 spectral intensities [27].

15

16 2.3.2. Reduced and total thiols determination by OPA-AE method

17 In this paper, the OPA-AE method was adapted and optimized for microplate analysis [28] and
18 analytical protocol was as follows: 15 μ L of sample or standard solutions was dispensed into
19 the wells of the microplate, where 145 μ L of a reagent solution (AE 26 mM and DTPA 20 mM
20 in 0.05 M borate buffer pH 9.9) and 130 μ L of OPA 50 μ M in ethanol were then added. Reduced
21 thiols determination was carried out by fluorescence intensity measurement after shaking for 7
22 min, with wavelengths set at 330 nm and 455 nm for excitation and emission respectively.

23 The analytical protocol was also adapted to total thiols measurement: during the first step, 15
24 μ l of sample was first mixed with 15 μ l of TCEP 20 mM. After a shaking time of 6 min, the
25 analytical protocol was continued as described in the previous paragraph.

1 Inorganic sulfides do not interfere in thiol compounds measurement with this OPA-AE method
2 (Fig. S2).

3

4 2.3.3. Reduced thiols determination by Ellman's reagent

5 The determination of reduced thiols was also performed using Ellman's method adapted to a
6 microplate measurement. Briefly, 100 μ l of sample or standard solution was mixed with 100
7 μ L of a DTNB reagent (1mg/ml in 200 mM phosphate buffer pH 8). The absorbance was
8 measured at 412 nm after incubation for 15 minutes.

9

10 **3. Results and discussion**

11 3.1. Thiols and sulfides derivatization

12 As displayed in Supplementary data (Fig. S1), the maximum absorption wavelength of the
13 products either formed between EP and a thiol compound such as mercaptopropionic acid
14 (MPA) or with sulfide ions is found at 288 nm and 304 nm, respectively. These wavelengths
15 have been selected for the course of this study. We deliberately chose MPA as the reference
16 thiol compound herein because this compound has an important environmental significance
17 [29] and possesses one of the higher pKa of thiol compounds [25], therefore requiring higher
18 pH value than other thiols to reach optimal reaction yield (reaction scheme between MPA and
19 EP is presented in Fig. S3).

20

21 3.1.1. pH optimization

22 Thiols derivatization with EP is usually carried out at basic pH to increase the reaction rate [30].
23 In the same time, it is well known that EP can react with other nucleophiles such as amines to
24 yield UV absorbing adducts [25,31]. Although these interfering compounds usually react
25 slowly with EP, they can potentially cause serious interferences in the case of environmental

1 samples with a wide range of concentrations for these compounds. Careful choice of the pH
2 value during derivation step can limit these interferences.

3 Fig. 1 displays the evolution of absorbance of EP derivatives as a function of pH value, formed
4 with i) MPA for reference thiol compound, ii) hydrogen sulfide and iii) glycine as reference
5 amine compound.

6 The absorbance of MPA derivative was stable at pH above 10, which is consistent with the
7 acidity of the thiol group for MPA ($pK_a = 10.3$) that allows reaction of MPA with EP through
8 its thiolate group. Regarding sulfides, the optimum pH for the reaction was between 9 and 9.5,
9 which suggests that EP reacts with the HS^- form of sulfides. Finally, as expected, glycine shows
10 higher response at pH around 10.5.

11 In this study, a pH value of 9.5 was thus selected as the best compromise for maximizing the
12 response of sulfides and thiols and minimizing interference of amines.

13

14 3.1.2. Effect of EP concentration and reaction time

15 EP concentration has obviously a significant influence on the reaction yield. The effect of EP
16 concentration was studied in the 1-75 mM range for MPA and hydrogen sulfide at pH 9.5. The
17 experimental results are shown in Fig. 2 and Fig. 3.

18 EP concentration greater than 10 mM had no significant impact on the derivatization yield of
19 MPA (Fig. 2), as demonstrated in previous studies concerning other thiols of biological or
20 environmental interest [25,26,32]. On the other hand, it can be seen that absorbance of the EP
21 derivative with HS^- is highly dependent on the concentration of EP. Hydrogen sulfide needs a
22 large excess of EP to reach significant yields, probably due to the fact that hydrogen sulfide has
23 a lower nucleophilicity than thiols. A concentration of 50 mM of EP was thus the best
24 compromise for the derivatization of MPA and sulfides and was selected for further

1 experiments. Glycine response was also tested at this concentration of EP and was still very
2 low (absorbance < 0.05).

3 Time-dependence of derivatization has been assessed by acquiring absorbance *versus* time
4 during the reaction between EP and MPA or hydrogen sulfide. Experiments were carried out
5 with a 50 mM EP solution (Fig. 3). Optimal reaction times were 2 minutes for the derivatization
6 of MPA (thereby limiting the cross interference of sulfides) and 15 minutes for the
7 derivatization of sulfides and were therefore selected for the following experiments.

8

9 3.1.3. Oxidized thiols

10 It is well known that thiol group can easily complex a wide range of metals in environmental
11 samples. Trace metals such as Cu^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} and Zn^{2+} have particularly high affinity
12 for SH groups, and we noticed that some complexes between thiols and metals almost
13 completely quenched subsequent reaction with EP (Cu^{2+} and Fe^{3+} especially). Moreover, thiols
14 can also be oxidized and form disulfide bond, which cannot be detected by direct reaction with
15 EP. To quantify total thiols content of a sample, the analytical protocol should therefore include
16 a reducing step to unbind metals from thiols or cleave disulfide bonds. Dithiothreitol (DTT) is
17 one of the most used agent for reducing thiols. Its use is however limited because DTT is not
18 stable due to air oxidation and must be kept cool and under an inert atmosphere. In recent years,
19 TCEP was used as thiols reducing agent [32,33] and offered many advantages such as an
20 improved stability in aqueous solution and a reactivity in a wider pH range than DTT.

21 Preliminary studies on quantification of MPA when bound with Cu^{2+} ions showed that TCEP
22 alone was insufficient to correctly quantify the concentration of MPA due to 45% yield of bond
23 cleavage (Fig. S4). Quantification could only be achieved after a reduction step by TCEP in the
24 presence of DTPA as complexing agent with a release of 97% of free MPA (Fig. S4). In this
25 sense, some experiments were carried out in order to optimize amount concentration of TCEP

1 during reduction step while a concentration of 2 mM DTPA was kept constant. The reducing
2 efficiency of TCEP was evaluated by spiking a 50 μM MPA solution with 160 μM Cu^{2+} .
3 Increasing concentrations of TCEP were added to the spiked MPA solution in the presence of
4 2 mM DTPA and left to react 6 minutes (Fig. 4.A). Experimental results showed that more than
5 97% of MPA was released within 6 minutes with a TCEP amount concentration between 20
6 and 40 mM. Reaction yield of reduction by TCEP was estimated by acquiring absorbance of
7 EP-MPA derivative as a function of time when reduction carried out with 20 mM TCEP and 2
8 mM DTPA (Fig. 4.B). A reaction time of 8 minutes was sufficient to quantify total MPA
9 content. Some experiments were also conducted to optimize the concentration of DTPA. The
10 results showed that a concentration of 2 mM DTPA was sufficient to effectively bind Cu^{2+} and
11 a higher DTPA concentration resulted in an increase of blank signal. A final concentration of
12 20 mM TCEP and 2 mM DTPA with a reaction time of 8 minutes were selected for final
13 analytical protocol and further experiments.

14

15 3.2. Analytical parameters

16 The analytical parameters were defined using the optimal analytical protocols. The calibration
17 curve obtained for MPA determination was $\text{Absorbance} = (0.006 \pm 0.0001)C + (0.0267 \pm 0.003)$,
18 where Absorbance was measured at 288 nm and C was the MPA concentration in μM
19 ($R^2=0.9986$; $n=7$ triplicates). Calibration curve was linear on the range 0-500 μM . The
20 calibration curve obtained for sulfides determination was $\text{Absorbance} = (0.0105 \pm 0.0004)C +$
21 (0.0105 ± 0.005) , where Absorbance was measured at 304 nm and C was the sulfide
22 concentration in μM ($R^2=0.9962$; $n=7$ triplicates). Calibration curve was linear on the range 0-
23 250 μM . The detection limits (3σ , $n=10$) were 0.6 and 2.8 μM (63.7 and $92.4 \mu\text{g.L}^{-1}$) for MPA
24 and sulfide respectively and the quantification limits (10σ , $n=10$) were 2.1 μM ($222.9 \mu\text{g.L}^{-1}$)
25 for MPA and 7.3 μM ($240.9 \mu\text{g.L}^{-1}$) for sulfide. The relative standard deviations (RSD) were

1 calculated from 10 successive standard determinations (100 μM) and reproducibility of 1.8%
2 and 1.9% was obtained for MPA and sulfide respectively.

3 In Table 1, main analytical features of our analytical protocols and previously developed
4 methods are compared. The present work shows that the developed method has a detection limit
5 among the lowest with a 2 minutes reaction time compared to 5 to 60 minutes for other methods.
6 NAM method [13] has a similar detection limit (1 μM) but requires a 60 minutes reaction time
7 and the response of various thiols is not uniform. The method also suffers from interference of
8 sulfites [34]. PAPAINE method [15] has the best detection limit and sensitivity but requires
9 degassing of solutions beforehand for 1 hour. The 4-DPS method displays improved reactivity
10 and sensitivity compared to the Ellman's method with a limited reaction time [12]. However, it
11 does not differentiate thiols and sulfides. The OPA-AE method adapted from the study of
12 Mopper and Delmas [8] has better analytical features but has the major drawback, as the NAM
13 method, to present a very variable reactivity for the different thiols (Fig. 5). Fig. 5 depicts the
14 relative reactivity of various thiols based on MPA as a reference. The EP method displays good
15 homogeneity in the response of the various thiols, like Ellman's method, while the OPA-AE
16 method displays a rather poor response for thiols such as GSH or MAA. The homogeneity of
17 the signals for various thiols enables general application of our methodology to quantify the
18 overall thiols.

19 For sulfides, the methods based on redox reactions are fast but suffers from interferences from
20 other reducing compounds such as nitrites [17]. Three methods compared in Table 1 present
21 fairly similar analytical performance. The advantage of the EP method developed here is to
22 separately quantify thiols and sulfides with the same derivatizing reagent in 15 minutes.

23

24 3.3. Interferences

1 The potential interference of other compounds on the determination of MPA and sulfide by the
2 proposed methods was studied. Series of solutions containing 100 μM of MPA or 100 μM of
3 sulfide were prepared with addition of potential interfering compounds selected for their
4 presence in environmental samples. It has been assumed that an element did not interfere when
5 the variation of resulting concentration of MPA or sulfide was lower than $\pm 10\%$ (Table 2).
6 We first evaluated the cross interferences between MPA and sulfides. Thanks to the spectral
7 deconvolution (see 2.3.1), each compound can be determined selectively in the presence of a
8 50-fold excess of the other analyte. We also evaluated the potential interferences resulting from
9 non-thiol sulfur compounds (reduced or oxidized) including methionine (in the range 0.5-3
10 mM), sulfates (20 mM) and sulfites (5 mM). Results indicate that these non-thiol compounds
11 do not lead to a significant change in the response of MPA or sulfides when they are present in
12 realistic ratios compared to targeted analytes, even for amino acids such as methionine or
13 glycine that could potentially lead to serious interference if experimental conditions were not
14 optimized. Other significant potential nucleophiles such as halides, thiocyanate or acetate (50
15 mM each) were also evaluated and displayed no interferences in the determination of MPA or
16 sulfides. Finally, interference from metals such as Cu^{2+} (160 μM), Fe^{3+} (180 μM) and Zn^{2+} (160
17 μM) was less than 6% with application of the reduction step with TCEP and DTPA.

18

19 3.4. Validation

20 Considering the possible matrix interference in real samples, determination of thiols and
21 sulfides in water samples was first validated by spiking real samples with different amounts of
22 MPA (for reduced thiols), cystine (for total thiols) and sodium sulfide (for dissolved sulfides).
23 This standard addition method has already been used in some studies about the determination
24 of thiol compounds [36,37]. The proposed method was applied to the determination of reduced
25 and total thiols and sulfides in raw and outlet wastewater spiked with 25 μM of MPA, cystine

1 and sodium sulfide. The recovery study of the targeted analyte was performed in triplicates.
2 Results were compared to recoveries obtained with Ellman's method. Results indicated a good
3 recovery of targeted analytes and an absence of matrix effect on the tested samples. The
4 recoveries of reduced thiols were $96.4 \pm 2.1\%$ in raw wastewater and $95.1 \pm 1.3\%$ in outlet with
5 developed method. Ellman's method gave recoveries of $96.1 \pm 1.9\%$ and $98.2 \pm 2.6\%$ for reduced
6 thiols on the same samples. The recoveries of total thiols were $98.3 \pm 1.6\%$ and $96.6 \pm 0.9\%$ in
7 raw wastewater and outlet respectively. The determination of sulfides showed also good
8 recoveries too with $98.5 \pm 1.7\%$ and $102.3 \pm 0.8\%$ in raw wastewater and outlet respectively.

9

10 **4. Conclusion**

11 The development of a microplate assay for the fast and individual determination of thiols and
12 sulfides has been successfully optimized in this study. Separated quantification of thiols and
13 sulfides has been achieved by exploitation of the absorbance shift of the derivatives of the
14 targeted analytes with ethyl propiolate. Total thiols can also be measured with an innovative
15 combination of a reducing agent (TCEP) and a complexing agent (DTPA). To the best of our
16 knowledge, this is the first method that can simultaneously quantify free thiols, total thiols
17 (bound to metals or in the oxidized form) and sulfides in aqueous samples, with a very
18 acceptable reaction time and low limits of detection. The method was successfully validated by
19 application to raw and treated wastewater and proved its applicability to real environmental
20 samples.

21

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25

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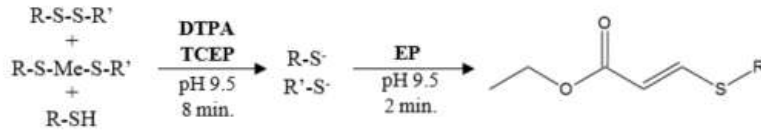
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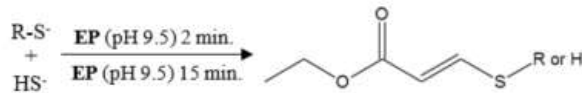
1 **Figures and Tables**

2

Total thiols determination



Reduced thiols and dissolved sulfides determination



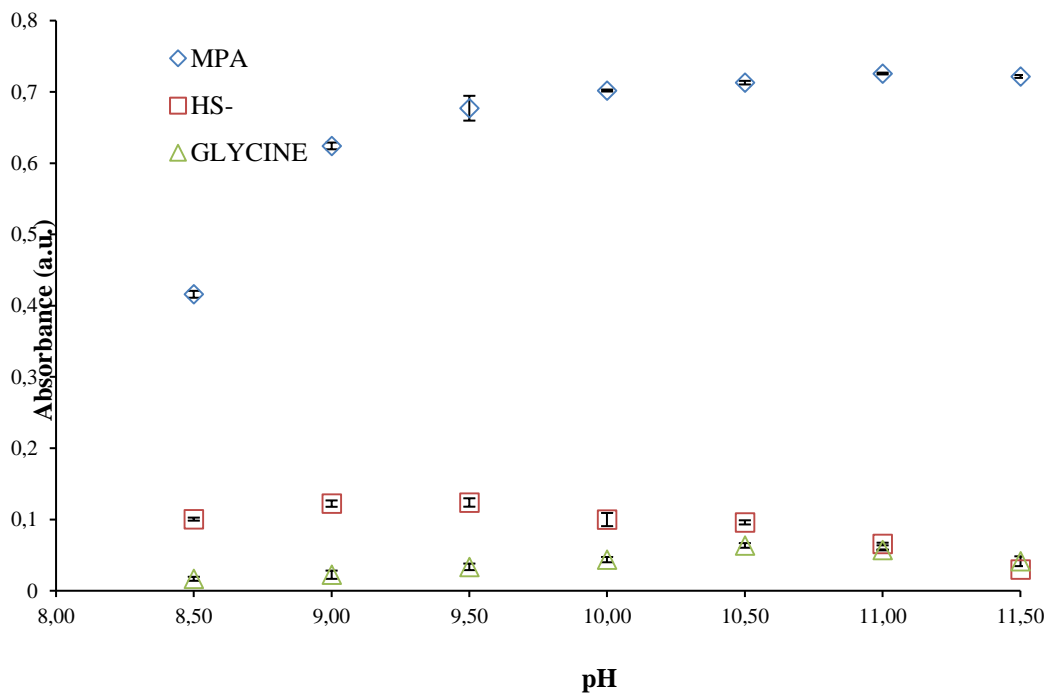
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4 **Scheme 1** Schematic depiction of the protocol for determination of total thiols, reduced thiols

5 and dissolved sulfides.

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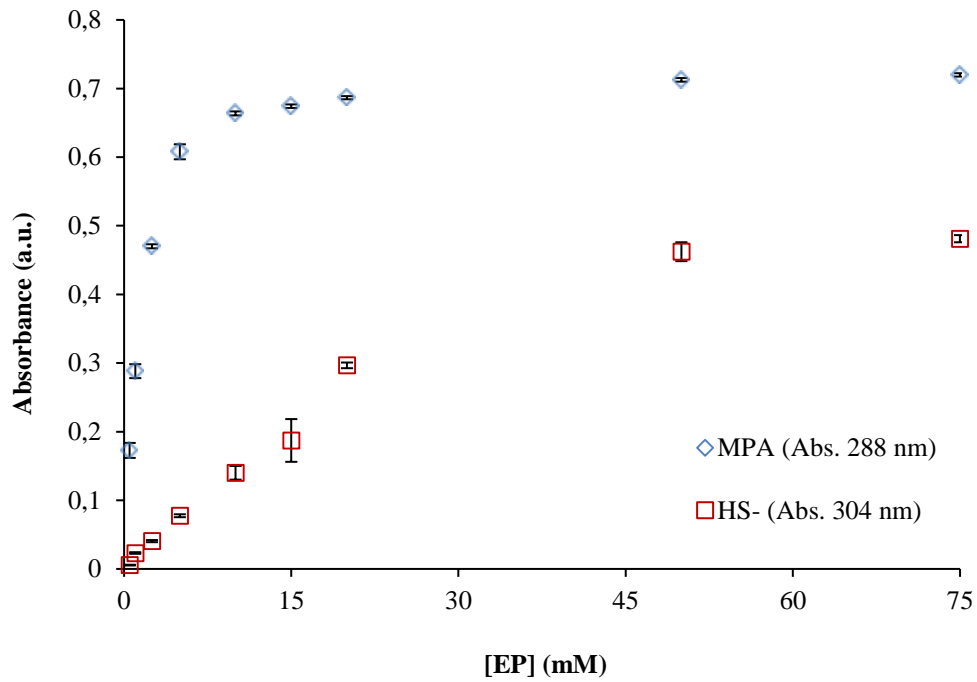
9 **Fig. 1** Evolution of absorbance (at 288 nm for MPA (diamonds) and glycine (triangles); at 304

10 nm for hydrogen sulfide (squares)) as a function of pH value during derivatization step. MPA,

1 HS⁻ and glycine were at 100 μM each. Derivatization was carried out in duplicate with EP at 5
2 mM during 5 minutes, pH was buffered by borate or phosphate.

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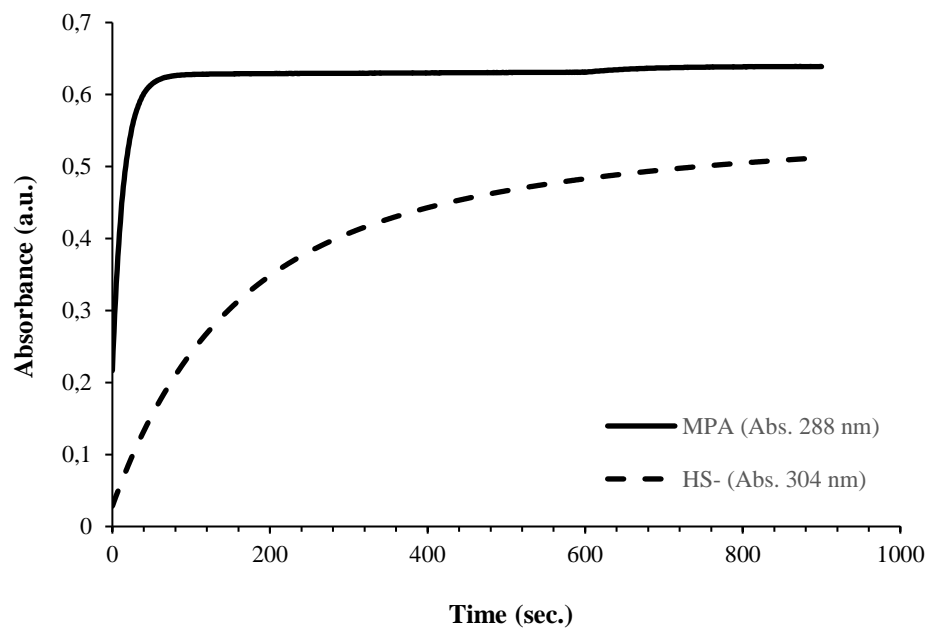


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6 **Fig. 2** Evolution of the absorbance (at 288 nm for MPA (diamonds); at 304 nm for HS⁻
7 (squares)) as a function of EP concentration. MPA and HS⁻ were at 100 μM each. Derivatization
8 was carried out in duplicate during 5 minutes, pH was buffered at 9.5 by borate.

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2 **Fig. 3** Evolution of absorbance (at 288 nm for MPA (solid line); at 304 nm for hydrogen sulfide
3 (dash line)) as a function of time. EP was at 50 mM. MPA and hydrogen sulfide were at 100
4 μM each. pH was buffered at 9.5 by borate.

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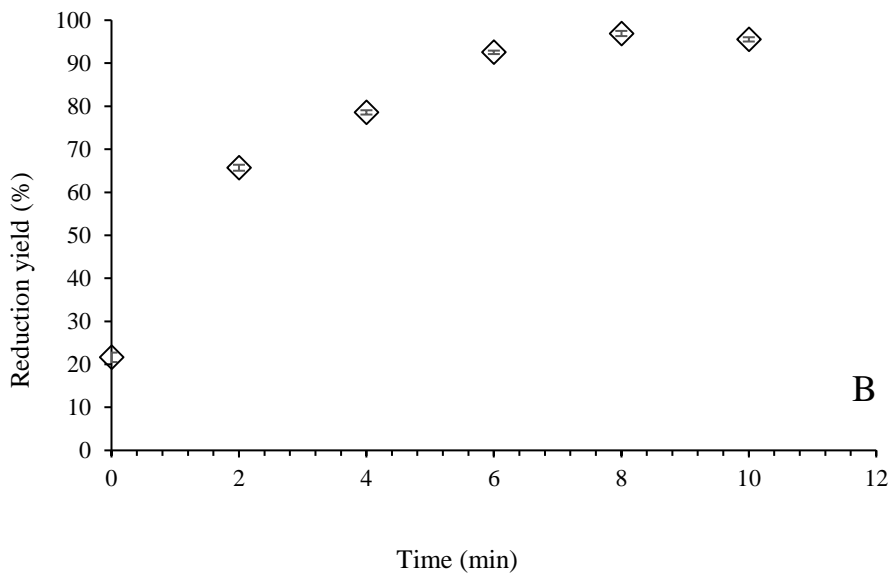
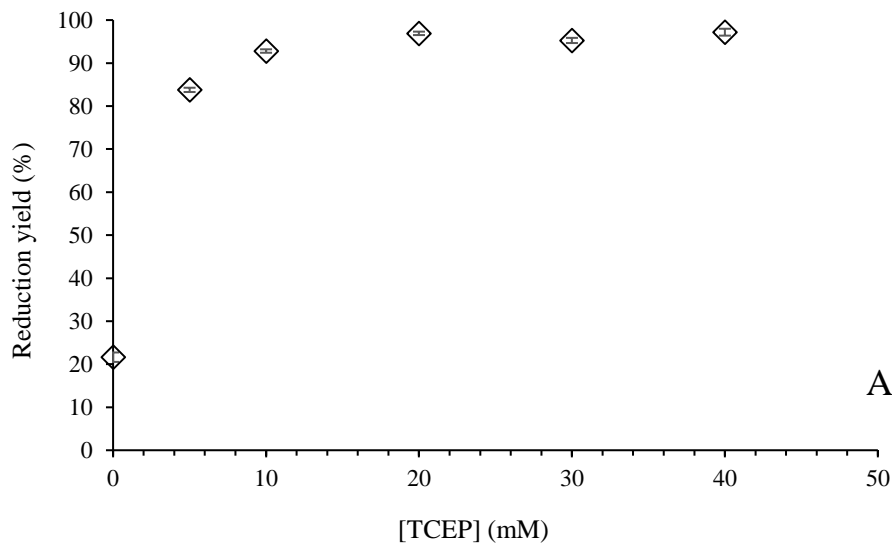
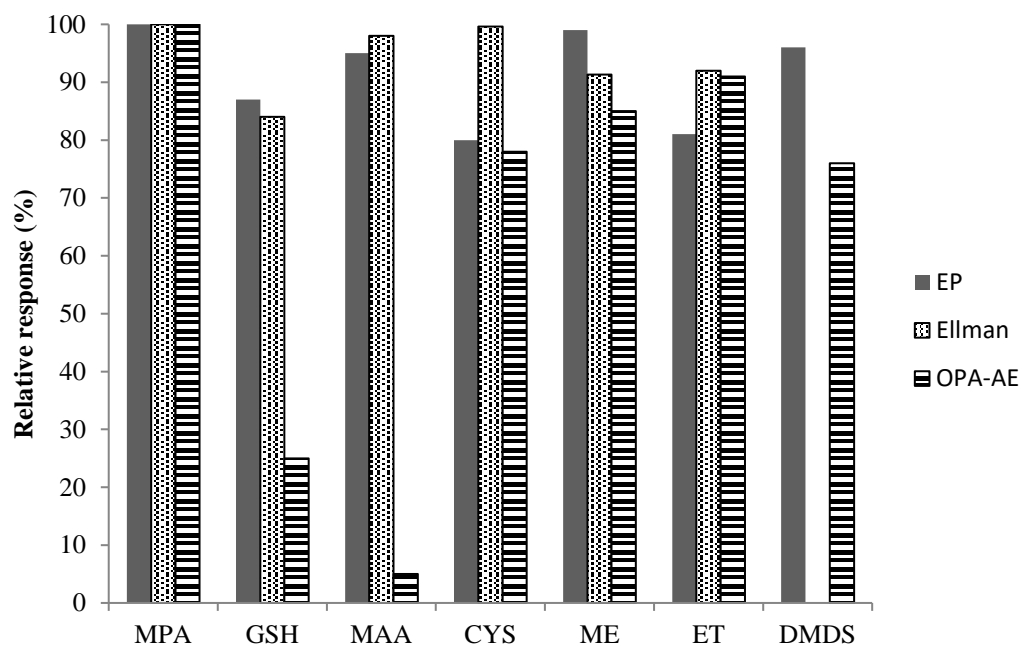


Fig. 4 Influence of various concentrations of TCEP with 6 min reduction time on reduction yield (Fig 4A); Influence of reduction time with 20 mM TCEP on reduction yield (Fig 4B).

Derivatization of 50 μM MPA in the presence of 160 μM Cu^{2+} was carried out in duplicate with 50 mM EP during 2 minutes, pH was buffered at 9.5 by borate.



1
2 **Fig. 5** Relative response of some thiol compounds compared to MPA (reference base 100) after
3 derivatization by EP following proposed method (grey plain), by Ellman's reagent (grey 25%)
4 or by OPA-AE method (horizontal lines). The concentration of tested thiols was 100 μ M each.
5 GSH: glutathione - OPA: Ortho-phtalaldehyde - AE: Aminoethanol – MAA: mercaptoacetic
6 acid – CYS: cysteine – ME: mercaptoethanol – ET: ethanethiol – DMDS: dimethyldisulfide

1 **Table 1** Main analytical features of some studies on free thiols and sulfides determination.

2 NAM: N-(9-acridinyl)maleimide – 4-DPS : 4,4'-dithiodipyridine – PAPAİN: cysteine protease – 2-PDS: 2,2' dipyridil disulfide

3

Analyte	Thiols						Sulfides		
Method (detection)	4-DPS (UV)	NAM (fluo)	PAPAİN (Vis)	DTNB (Vis)	OPA-AE (fluo)	EP (UV)	2-PDS (UV)	Fe(III) reduction (Vis)	EP (UV)
Standard	Mercaptans	GSH	Cysteine	GSH	MPA	MPA	Sodium sulfide	Sodium sulfide	Sodium sulfide
Range (μM)	3-40	1-100	0.2 – 1.5	1-450	1-200	1-500	1.8-360	1-42	3-250
Detection limit (μM)	3	1	0.2	1.1	0.2	0.6	1.8	1	2.8
Quantification limit (μM)	-	-	-	3.5	0.6	2.1	-	-	7.3
Reaction time (min.)	5	60	60	15	1	2	5	few min.	15
RSD (%)	-	-	-	6.2	3.1	1.8	-	2.5	1.9
Reference	[12]	[13]	[15]	[34]	Method adapted from [7] and [28]	This work	[16]	[17]	This work

4

1

2 **Table. 2** Recovery of MPA and sulfides in binary solution with potential interfering
 3 compounds. [EP] = 50 mM; [MPA] or [HS⁻] = 100 μM; pH = 9.5; Reaction time = 2 min. (MPA)
 4 or 15 min (HS⁻).

5

MPA (100 μM)			Sulfides (100 μM)		
Interfering compound	Amount	Recovery	Interfering compound	Amount	Recovery
Sulfates	20 mM	92.40%	Sulfates	20 mM	95.70%
Sulfites	5 mM	94.80%	Sulfites	5 mM	93.70%
Methionine	0.5 mM	95.20%	Methionine	3 mM	96.70%
Glycine	1 mM	93.50%	Glycine	1 mM	91.50%
Ammonium	20 mM	98.70%	Ammonium	20 mM	95.60%
Iodide	50 mM	98.30%	Iodide	50 mM	98.50%
Chloride	50 mM	97.90%	Chloride	50 mM	97.60%
Bromide	50 mM	98.90%	Bromide	50 mM	96.70%
Thiocyanate	50 mM	97.10%	Thiocyanate	50 mM	96.60%
Acetate	50 mM	98.90%	Acetate	50 mM	99.30%
Cu ²⁺ *	160 μM	98.10%	Cu ²⁺ *	160 μM	98.20%
Fe ³⁺ *	180 μM	94.30%	Fe ³⁺ *	180 μM	95.10%
Zn ²⁺ *	160 μM	99.60%	Zn ²⁺ *	160 μM	98.40%

6 * with TCEP 20 mM and DTPA 2 mM

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