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Determination of S-nitrosoglutathione in plasma: Comparison of two methods

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ABSTRACT

In this work we compared the results of the GSNO determination in human plasma by two independent methods. The first method is based on the pre-column derivatization of GSNO thiolic part by *p*-hydroxymercury benzoate (PHMB) and followed by the determination of GS-PHMB product by reversed phase chromatography coupled to chemical vapour generation atomic fluorescence spectrometry (RPC-CVGAFS). The second method is based on RPC separation of GSNO from interfering compounds and the post-column, on-line enzymatic hydrolysis of GSNO by commercial γ-glutamyl transferase (GGT) and fluorescence detection.

Endogenous GSNO was determined only in plasma from blood sampled by syringe (not by Vacutainers®) and ranged between 157 and 257 nM on the basis of RPC-CVGAFS method, and between 90 and 225 nM by RPC-FD method. There was a good correlation between the two methods (slope = 1.06 ± 0.09, R^2 = 0.9543). RPC-CVGAFS method based on PHMB derivatization determined a GSNO concentration 60 ± 20 nM in excess with respect to RPC-FD method. Sampling issues connected with common blood sampling procedures like venipuncture and sampling in syringe or Vacutainers® still introduce in GSNO analysis unknown factors, which require further investigations.

1. Introduction

Nitric oxide (NO) is an important messenger molecule that plays a critical role in both physiological and pathological vascular signalling. In blood vessels, it is synthesized by the endothelial isoform of the enzyme nitric oxide synthase (eNOS) [1,2]. Once synthesized, NO can diffuse in the underlying smooth muscle cells and promoting vasodilatation or diffuse in erithrocytes and reacts in a very rapid manner with oxyhemoglobin to form S-nitrosohemoglobin [3]. In plasma NO produced by eNOS can also be oxidized to peroxynitrite and nitrite in a reaction catalyzed by ceruloplasmin [4] to form N-nitrosamines and S-nitrosothiols (RSNO) [5].

S-nitrosoglutathione (GSNO) is considered a natural NO reservoir and a reactive nitrogen intermediate.

Recently, we published a paper on the determination of S-nitrosoglutathione (GSNO) and other nitrosothiols by a single step decomposition-derivatization process with an organic mercurial compound, *p*-hydroxymercury benzoate (PHMB) [6]. The product (GS-PHMB) was determined by reversed phase chromatography (RPC) coupled to chemical vapour generation atomic fluorescence spectrometry (CVGAFS). The method performance and the purportedly elevated GSNO levels reported were further discussed in two letters to the editor [7,8] and in a recent paper [9].

Excellent papers reviewed the issues related to nitrosothiols and nitrite determination in plasma and blood [10–13]. Method validation in this field is not trivial because of the absence of certified reference materials and/or consolidated analytical methods. In 2006 Wang et al. [14] claimed validation of industry standard triiodide-based chemiluminescence assay. Wang et al. reported that chemiluminescence assay does not underestimate RSNO levels. However, no cross-validation of the method was performed using method based on an analytical principal different that of validated method. On the other hand in 2007 Hausladen et al. reported on the performance of the triiodide assay *vs.* photolysis chemiluminescence in side-by-side assays of multiple nitrosylated standards of varied reactivity and in assays of endogenous Fe- and S-nitrosylated haemoglobin, concluding that the triiodide assay is strongly influenced by sample composition and reactivity and does not reliably identify, quantify, or differentiate NO species in complex biological mixtures [15].

In this work, for method evaluation we performed GSNO determinations and spike recovery experiments by two independent methods. GSNO determination was performed by RPC-CVGAFS

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method [6] and by a method, recently published, based on liquid chromatography separation coupled to on-line enzymatic hydrolysis of GSNO by commercial γ-glutamyl transferase (GGT) [16]. In RPC-CVGAFS method PHMB reacts directly with RSNOs without forming free thiols, giving stable RS-PHMB complex. Then the complexes are on line oxidized and Hg(II) converted to volatile Hg(0), which is detected by AFS. In the latter method (RPC-FD), RSNOs are separated in a RPC column coupled with a post-column reaction coil where the enzyme GGT allows the specific hydrolysis of the γ-glutamyl moiety of GSNO giving S-nitrosocysteinylglycine (GCNO). In the reaction coil GCNO is decomposed by copper ions giving oxidized cysteinylglycine and nitric oxide (NO). NO immediately reacts with 4,5-diaminofluorescein (DAF-2) forming a triazole derivative (DAF-2T), which is detected fluorimetrically (FD). Thus, the measurement principles of RPC-CVGAFS and RPC-FD are different and characterized by probable different sources of errors and biases.

Sampling issues are also discussed because the analyte stability in the pre-analytical step is probably the most critical part in RSNO determination. We recently highlighted the importance of controlling metal-catalyzed, enzyme-catalyzed decomposition reactions and transnitrosilation reactions [6]. We demonstrate herein that even the sampling material may affect for still unknown mechanisms GSNO stability.

2. Experimental

2.1. Chemicals

For RPC-FD methods GGT (G-8040, Type I crude from bovine kidney) and glycylglycine (GG, G-1002) were purchased from Sigma–Aldrich (Sigma, Chemical Co., Milan, Italy). Copper sulfate anhydrous (2791) was purchased from Merck (Laborchimica, Firenze, Italy). Five mM solution of 4,5-diaminofluorescein (DAF-2) solution in dimethyl sulfoxide (DMSO) (251510) and its triazole derivative (DAF-2T, 251510, 0.5 mM in DMSO) were purchased from Calbiochem (La Jolla, CA, USA).

For RPC-CVGAFS method PHMB (4-(hydroxymercuric) benzoic acid) and sodium salt (CAS No. 138-85-2, $HOHgC_6H_4CO_2Na$) was purchased from Sigma (Sigma–Aldrich, Chemical Co.). 1×10^{-2} M stock solution of PHMB was prepared by dissolving the sodium salt in 0.01 M NaOH in order to improve its solubility, stored at 4 °C, and diluted freshly, just before use. The precise concentrations of PHMB solutions were determined from the absorbance at 232 nm ($\varepsilon_{232} = 1.69 \times 10^4\ cm^{-1}\ M^{-1}$).

Stock solutions of GSH (G6529), cysteine (30089, Cys) and homocysteine (H4628, HCys) (Fluka-Sigma–Aldrich, Milan, Italy) were prepared in 0.1 M phosphate buffer solution (PBS) pH 7.4, 0.5 mM ethylendiaminotetracetic acid (EDTA). In order to prevent oxidation, standard solutions of thiols were prepared daily and kept cold (4 °C) and protected from light until used.

Stock solution of GSNO (N4148, CAS No. 57564-91-7, Sigma, Chemical Co., St. Louis, MO, USA) was prepared in 0.1 M, PBS pH 7.4, 0.5 mM EDTA. In these experimental conditions (0.1 M, PBS pH 7.4, 0.5 mM EDTA), in the absence of PHMB added, GSNO standard solution was stable during the working day (9 h time tested at room temperature). Aliquots of stock solution were prepared and stored at −20 °C until used. The concentration of GSNO was calculated from absorbance at 334 nm using the extinction coefficient 977 $M^{-1}\ cm^{-1}$ [17]. At −20 °C GSNO stock solution was stable for about 1 month.

S-nitrosocysteine (CysNO) and S-nitrosohomocysteine (HCysNO) were prepared by reacting 1 M $NaNO_2$ in H_2O with 1.1 M thiols in 0.5 M HCl, 0.5 mM EDTA at 0 °C for 40 min [18]. RSNOs were diluted in 0.1 M, PBS pH 8.0, 0.5 mM DTPA and stability was tested before any other use.

N-ethylmaleimide (NEM, 04259 BioChemika Ultra, ≥99.0%), DL-serine (84980), boric acid (B-0252) and diethylentriaminepentaacetic acid pentasodium salt (DTPA, 17969) were purchased from Fluka (Fluka GmbH, Buchs, Switzerland). 50 mM stock solution of ethylenediaminetetracetic acid (405501, EDTA disodium salt, RPE) was purchased from Carlo Erba (Milan, Italy). Phosphate buffer solution (PBS) was prepared from monobasic monohydrate sodium phosphate and dibasic anhydrous potassium phosphate (BDH Laboratory Supplies, Poole, England).

Fresh 0.4 M NEM stock solution was prepared in MilliQ water daily. 0.4 M serine/borate complex (SBC) stock solution was prepared in 1 M, PBS pH 8.0. GGT was prepared in 0.1 M, PBS pH 8.0. 0.2 M GG stock solution was prepared in 1 M, PBS pH 8.0. $CuSO_4$ was prepared in MilliQ water.

The buffer solutions were prepared from monobasic monohydrate sodium phosphate and dibasic anhydrous potassium phosphate (BDH Laboratory Supplies, Poole, England).

L(+)-Ascorbic acid sodium salt (11140 BioChemika, ≥99.0% NT, CAS No. 134-03-2) and N-ethylmaleimide (NEM, 04259 BioChemika Ultra, ≥99.0%, Fluka) were purchased from Sigma and the stock solution prepared daily in MilliQ water.

Methanol for RPLC was purchased from Carlo Erba (Rodano, MI, Italy).

Stock solutions of $NaBH_4$ (about 6.5 M) was prepared by dissolving the solid reagent (Merck, pellets, reagent for AAS, minimum assay >96%) into 0.3% (m/v) NaOH solution. The solutions were microfiltered through a 0.45 μm membrane and stored in a refrigerator. Dilute solutions of $NaBH_4$ (0.05 M) were prepared by appropriate dilution of the stock solutions, with the total NaOH concentration maintained at 0.3% (m/v).

The 24–26% hydrazine standard solution (53847, CAS No. 10217-52-4) was purchased from Fluka Chemie and the optimized concentration (0.1 M) added to $NaBH_4$ solution containing 0.3% (m/v) of NaOH.

3.5 M HCl solutions were prepared with 37% (m/m) HCl (Carlo Erba)

A working solution of Br^-/BrO_3^- was prepared by solid reagents (Carlo Erba) (0.075 M Br^-, 0.015 M BrO_3^-) keeping an approximate Br^-/BrO_3^- 5:1 molar ratio on the basis of stoichiometry of redox reaction. Addition of a moderate excess of Br^- guaranteed a complete conversion of bromate to Br_2.

Water deionized with a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

Safety considerations: PHMB is toxic. Inhalation and contact with skin and eyes should be avoided. All work should be performed in a well-ventilated fume hood.

2.2. Human blood sampling

Fourteen venous blood samples were collected by venipuncture with needle connected by silicon tubing with Vacutainers®, a common, safe, blood sampling method. 0.5 mM EDTA/12 mM N-ethylmaleimide (NEM)/10 mM serine borate complex (SBC) were added into Vacutainers® containing heparin to avoid RSNO decomposition, as previously reported [6,16]. Five blood samples were collected from volunteer donors by venipuncture with a polyethylene (PE) syringe and placed into containers containing heparin/0.5 mM EDTA/12 mM NEM/10 mM SBC. The latter was the same sampling method adopted for blood sampling in Ref. [6]. Blood samples were processed as previously reported [6] and plasma ultrafiltrate analysed by both methods. Briefly, after low-speed centrifugation (1500 × *g*, 10 min) at room temperature, plasma samples were diluted 1:1 in 0.1 M, PBS pH 7.4, 0.5 mM EDTA, loaded onto the sample reservoir of an Amicon Microcon YM-3 centrifugal filter units (cut-off 3000 Da; Millipore, Bedford, MA, USA) and centrifuged at 11,000 × *g* for 90 min at 4 °C, to remove proteins

and high molecular weight compounds. Ultrafiltrates were then injected in the two chromatographic systems. For the experiments of recovery GSNO was added to plasma before ultracentrifugation.

2.3. Procedures

For RPC-CVGAFS method the procedure for the simultaneous derivatization/decomposition of GSNO and other RSNOs by PHMB/ascorbate was described in detail elsewhere [6]. Briefly, in quantitative determinations RSNO standard solutions or plasma ultrafiltrates were treated with 150 μM PHMB and 3.15 mM ascorbate at room temperature (21 ± 1 °C) for 30 min before injection.

In RPC-FD method RSNO standard solutions or plasma ultrafiltrate were directly injected in the chromatographic system.

2.4. Calibrations

For the calibration experiments of thiols in RPC-CVGAFS, GSH, Cys and HCys were derivatized by diluting the stock solution in 0.1 M, PBS (pH 7.43) containing a stoichiometric amount or a moderate excess of PHMB, at 25 °C. After a reaction time ≥ 5 min at room temperature (21 ± 1 °C), the solutions were injected in the RP chromatographic column.

For the calibration of RSNOs in RPC-CVGAFS, RSNOs were derivatized as reported in Section 2.3 and injected in the RP chromatographic column. The yield of the derivatization was evaluated by comparing the slope of the calibration curves of RSNO with the calibration curve of the corresponding thiol.

In RPC-FD method calibrations were performed directly injecting RSNO standard solutions at different concentrations in RPC-FD system.

2.5. Instrumental set-up

For HPLC-FD method the HPLC unit consisted of a pump (P4000, ThermoQuest) equipped with a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA), a 100 μL poly(etheretherketone) (PEEK tubing, Upchurch, Oak Harbor, WA) injection loop and an HPLC column (Gemini RP C_{18} 250 mm × 4.6 mm, silica particle size 5 μm, equipped with a Guard Cartridge KJ0-4282, Phenomenex). The eluent for the chromatographic analysis was 99% 0.02 M, PBS pH 6.0/1% methanol (99:1, v/v), flowing in the optimized conditions at 0.8 mL/min. The flow eluting from HPLC column was monitored between 210 and 650 nm by a UV–visible diode array detector (DAD) equipped with a 5 cm path length flow cell (UV6000, ThermoQuest). The effluent was blended in a low dead-volume mixing-tee with the reaction solution delivered by a second HPLC pump (Jasco PU2080) at a flow rate of 0.3 mL/min, containing 140 mU/mL GGT, 0.03 mM copper sulfate, 1.2 mM GG and 0.37 μM DAF-2 in 0.1 M, PBS pH 8.0 (optimized conditions, concentrations in the reaction coil). The enzymatic reaction proceeded in the optimized conditions in a PFA reaction coil of 2.8 mL, corresponding to a $t_{reaction}$ = 2.55 min, kept at the constant temperature of 37 °C in a water bath [16]. Thus, the DAF-2T signal was specifically detected by a fluorescence detector (FL3000, ThermoFinnigan) operating at excitation wavelength λ_{ex} = 486 nm and emission wavelength λ_{em} = 516 nm.

For RPC-CVGAFS method the HPLC unit was a gradient pump (P4000, ThermoQuest) equipped with a Rheodyne 7125 injector (Rheodyne, Cotati, CA, USA), and a 50 μL injection loop. The separations were carried out by a reversed phase HPLC column Hydra RP C_{18} (Phenomenex) 250 mm × 4.6 mm (silica particle size 4 μm), equipped with a Guard Cartridge KJ0-4282 Phenomenex with an isocratic elution in 99% 0.02 M, PBS pH 6.0, 1% methanol, flowing at 1 mL/min. Sample eluted from the column passed into a diode array detector (DAD, UV6000, ThermoQuest) equipped with a 5 cm path length flow cell (10 μL), and finally into the CVGAFS detection system. The GS-PHMB complex can be determined by CVAFS detection in the adopted operating conditions with a detection limit of 25 nM, a precision (CV%) of 6.5% at 0.3 μM concentration level, and a 0.08–50 μM linear dynamic range.

All the solutions were filtered by a 0.45 μm cellulose acetate filter (Millipore).

The continuous flow (CF) mercury chemical vapour generator modified for on line oxidation of organic mercury to inorganic Hg(II) in a miniaturized Ar/H_2 flame was described in details elsewhere [6]. Reagent concentrations, reaction coil dimension, and flow rates, as well, were optimized and reported elsewhere [19].

Table 1
RSNO determination in 14 plasma ultrafiltrates by RPC-CVGAFS. Blood from volunteer donors was sampled in Vacutainers® containing heparin/0.5 mM EDTA/12 mM NEM/10 mM SBC. No CysNO and HcysNO could be detected by RPC-FD because of interferences. No GSNO could be detected by either instrumental method in these samples.

Sample number	CysNO (nM)	HcysNO (nM)
1	1910	1490
2	1570	739
3	1950	884
4	1840	916
5	876	664
6	960	629
7	1000	1280
8	642 ± 50[a]	482 ± 78[a]
9	608	197
10	652	428
11	1026	742
12	710	452
13	526	764
14	1867 ± 153[a]	1817 ± 104[a]

[a] SD on N = 3 replicates.

3. Results and discussion

Table 1 reports the results of RSNO determination in 14 plasma ultrafiltrates by RPC-CVGAFS. No CysNO and HcysNO could be detected by RPC-FD because of interferences of ascorbic, dehydroascorbic and uric acid [16]. No GSNO could be detected by either instrumental method in these samples. These results indicate that no GSNO was detected by either of the two methods in samples obtained by Vacutainers®, only CysNO and HcysNO were found by RPC-CVGAFS method.

To perform a cross-validation of the two methods several plasma samples and pooled plasma samples among those reported in Table 1 were spiked with various concentrations of GSNO in the 100–2150 nM range and analysed by both methods. Fig. 1 shows the correlation plot of RPC-CVGAFS method *vs.* RPC-FD enzymatic method.

GSNO was also determined by both methods in plasma ultrafiltrates from blood sampled by venipuncture with polyethylene (PE) syringe and placed into containers containing heparin/0.5 mM EDTA/12 mM NEM/10 mM SBC (N = 5 volunteer donors) (Table 2). Table 2 reports also data obtained for CysNO and HCysNO by RPC-CVGAFS method. Fig. 2 shows the correlation plot of GSNO determination by RPC-CVGAFS *vs.* RPC-FD method obtained in N = 5 samples and standard addition to sample No. 1.

There is a good correlation between the two methods. However, RPC-CVGAFS method based on PHMB derivatization determined a GSNO concentration 60 ± 20 nM in excess with respect to RPC-FD method based on GGT/Cu(II)-mediated decomposition of GSNO and fluorescence detection of NO released. This result can be explained by two hypothesis. The first is the involvement of a currently unknown source of GSH different than GSH, GSSG, GSSR. Likely this hypothesis can be excluded on the basis of results reported

Table 2
RSNO determination in 5 plasma ultrafiltrates. Blood from volunteer donors was sampled by venipuncture with PE syringe and placed into containers containing eparine/0.5 mM EDTA/12 mM NEM/10 mM SBC. GSNO was spiked to plasma No. 1.

Sample number	CysNO (nM) PHMB, RPC-CVGAFS	HcysNO (nM) PHMB, RPC-CVGAFS	GSNO (nM) PHMB, RPC-CVGAFS	GSNO (nM) Enzymatic RPC-FD
1	1070 ± 14[a]	1110 ± 71[a]	225 ± 22[a] (242 ± 17, R^2 = 0.9836)	136 ± 15[a] (134 ± 15, R^2 = 0.9875)
1 + 50 nM GSNO	1180	980	314	189
1 + 150 nM GSNO	1005	950	466	369
1 + 300 nM GSNO	970	1010	630	548
2	656	489	157	90
3	2323	1677	218	180
4	1000	840	173	150
5	1390	779	277	225

Between brackets we report the values calculated by standard addition curve.
[a] SD on $N = 3$ replicates.

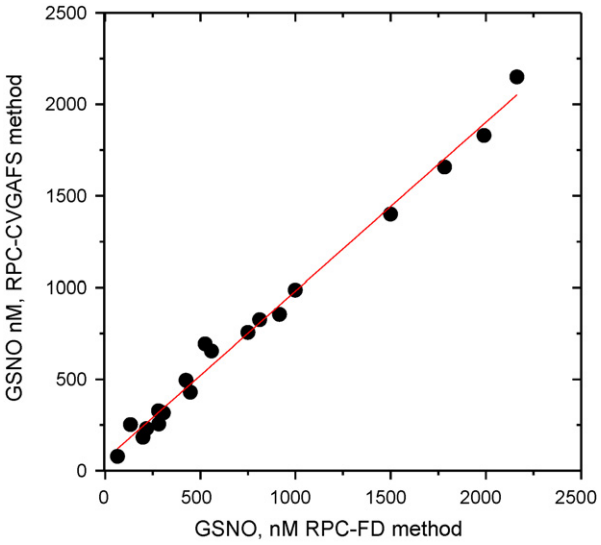

Fig. 1. Correlation plot of RPC-CVGAFS method *vs.* RPC-FD enzymatic method (intercept = 60 ± 23 nM; slope = 0.92 ± 0.02; R^2 = 0.9889). Data obtained by spiking several plasma and pooled plasma samples of Table 1 with GSNO in the 100–2150 nM range.

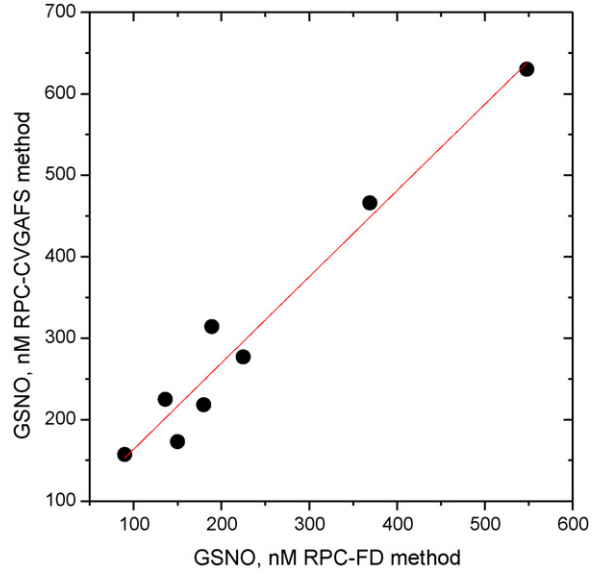

Fig. 2. Correlation plot for plasmatic GSNO concentration obtained for blood sampling by venipuncture with PE syringe. GSNO determined by RPC-CVGAFS *vs.* RPC-FD method ($N = 5$ different samples and $N = 3$ standard addition to plasma No. 1; intercept = 58 ± 24 nM, slope = 1.06 ± 0.09; R^2 = 0.9543).

in Table 1 and of all topics previously discussed [6]. The lack of peaks with the retention time of GS-PHMB derivative in the ultrafiltrate of the unspiked blood samples of Table 1, suggests that neither NEM-complexed GSH nor GSSG nor RSR compounds (by analogy with NEM-complexed thiols) present in the plasma ultrafiltrate interfere with the measurement of GSNO by this method. The second hypothesis could be related to a different speciation of GSNO spiked and endogenous GSNO, which could be responsible for a slower kinetics of the on line GSNO decomposition process mediated by GGT. We excluded an appreciable degradation of GSNO in the column by performing elution with and without EDTA in the eluent phase. Calibration curves of GSNO in both cases gave non-significant differences.

4. Concluding remarks

Endogenous GSNO was determined only in plasma from blood sampled by PE syringe and ranged between 157 and 257 nM on the basis of RPC-CVGAFS method, according to previous data [6], and between 90 and 225 nM by RPC-FD method. CysNO and HCysNO were present in all samples independent from the sampling procedure and were determined by RPC-CVGAFS. This result shows that in the sampling conditions adopted in this study the stability of CysNO and HCysNO was controlled. However, GSNO stability in the pre-analytical step still suffers for additional, unknown factors, which require further investigations before considering GSNO as a good bio-marker.

To the best of our knowledge, for the first time two independent analytical methods run side-by-side were able to give comparable results for the GSNO determination in human plasma. This represents a major improvement with respect to the orders of magnitude discrepancies reported in the literature and makes currently the described methods “more appropriate” than others [9]. These findings could also indicate that the critical step in GSNO determination in plasma is not the measurement protocol, but the method adopted for sampling blood.

References

[1] R.F. Furchgott, J.V. Zawadzki, Nature 288 (5789) (1980) 373–376.
[2] R.M. Palmer, A.G. Ferrige, S. Moncada, Nature 327 (6122) (1987) 524–526.
[3] X. Liu, M.J.S. Miller, M.S. Joshi, H. Sadowska-Krowicka, D.A. Clark, J.R. Lancaster Jr., J. Biol. Chem. 273 (30) (1998) 18709–18713.
[4] S. Shiva, X. Wang, L.A. Ringwood, X. Xu, S. Yuditskaya, V. Annavajjhala, H. Miyajima, N. Hogg, Z.L. Harris, M.T. Gladwin, Nat. Chem. Biol. 2 (9) (2006) 486–493.
[5] K. Inoue, T. Akaike, Y. Miyamoto, T. Okamoto, T. Sawa, M. Otagiri, S. Suzuki, T. Yoshimura, H. Maeda, J. Biol. Chem. 274 (38) (1999) 27069–27075.
[6] E. Bramanti, K. Jacovozzi, L. D'Ulivo, C. Vecoli, R. Zamboni, Z. Mester, A. D'Ulivo, Talanta 77 (2008) 684.
[7] D. Tsikas, Talanta 79 (2009) 552–553.
[8] E. Bramanti, V. Angeli, Z. Mester, A. D'Ulivo, Talanta 79 (2009) 554.
[9] B.W. Allen, J.S. Stamler, C.A. Piantadosi, Trends Mol. Med. 15 (2009) 452–460.
[10] A. Gow, A. Doctor, J. Mannick, B. Gaston, J. Chromatogr. B 851 (2007) 140–151.
[11] L.A. Palmer, B. Gaston, Methods Enzymol. 440 (2008) 157–176.

[12] M.M. Pelletier, P. Kleinbongard, L. Ringwood, R. Hito, C.J. Hunter, A.N. Schechter, M.T. Gladwin, A. Dejam, Free Rad. Biol. Med. 41 (2006) 541–548.
[13] J.S. Stamler, Circ. Res. 94 (2004) 414–417.
[14] X. Wang, N.S. Bryan, P.H. MacArthur, J. Rodriguez, M.T. Gladwin, M. Feelisch, J. Biol. Chem. 37 (2006) 26994.
[15] A. Hausladen, R. Rafikov, M. Angelo, D.J. Singel, E. Nudler, J.S. Stamler, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 2157.
[16] E. Bramanti, V. Angeli, M. Franzini, C. Vecoli, R. Baldassini, A. Paolicchi, R. Barsacchi, A. Pompella, Arch. Biochem. Biophys. 487 (2009) 146.
[17] K.R. Matthews, S.W.J. Kerr, J. Pharmacol. Exp. Ther. 267 (1993) 1529.
[18] A. Doctor, R. Platt, M.L. Sheram, A. Eischeid, T. McMahon, T. Maxey, J. Doherty, M. Axelrod, J. Kline, M. Gurka, A. Gow, B. Gaston, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 5709–5714.
[19] A. D'Ulivo, E. Bramanti, L. Lampugnani, R. Zamboni, Spectrochim. Acta B 56 (2001) 1893.