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Determination of 3-mercaptopropionic acid by HPLC: A sensitive method for environmental applications

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ABSTRACT

The organic sulfur compound 3-mercaptopropionic acid (3-MPA) is an important thiol intermediate in organic sulfur metabolism in natural environments. It is generated during degradation of sulfur-containing amino acids (e.g. methionine) and from demethylation of dimethylsulfoniopropionate (DMSP). This pathway is an alternative enzymatic process in the DMSP catabolism that routes sulfur away from the climatically-active dimethyl sulfide (DMS). 3-MPA detection and subsequent quantification in different matrices is difficult due to its extreme reactivity. We therefore developed a sensitive method for determination of 3-MPA based on pre-column derivatization with monobromobimane and analysis by high-performance liquid chromatography (HPLC) with fluorescence detection. This methodology was first tested with 3-MPA standards under low (0.005–0.2 μmol L^{-1}) and high (1–25 μmol L^{-1}) concentrations. For the optimization of the reaction, CHES and, alternatively, Tris–HCl buffers were evaluated in the derivatization step, with Tris–HCl showing more effective separation of thiol derivatives and a better 3-MPA peak shape. The detection limit was 4.3 nmol L^{-1} with a 10 μL sample injection, and mean recoveries of 3-MPA ranged from 97 to 105% in estuarine waters with different salinities (0.17 and 35.9 ppt). The linearity ($r > 0.99$) and repeatability of detector response, with intra- and inter-day precision (% CV) of 2.68–7.01% and 4.86–12.5%, respectively, confirmed the reliability of the method. Previous 3-MPA analytical methods required immediate analysis due to unstable derivatives, but in this method we achieved high stability of the derivatized samples when stored at 4 °C, with only a 3–5% loss after more than one year of storage. This method was successfully applied to measure 3-MPA concentrations and rates of 3-MPA production in a variety of intertidal estuarine sediment slurries. Dissolved 3-MPA concentrations in these sediment slurries varied between 2 and 237 μmol L^{-1} and, 3-MPA net fluxes ranged in wet sediments between -3.6 ± 1.7 and 30 ± 5 μmol L^{-1} g^{-1} h^{-1}. Thus, the application of this optimized methodology showed an efficient performance for measuring 3-MPA in environmental samples, with a straightforward sample derivatization and a simple analysis of stable 3-MPA derivatives.

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1. Introduction

Low molecular weight thiols are important because of their biological turnover in natural environments. In fact, they have a fundamental role in sulfur biogeochemistry, being major intermediates in the processes involved in microbial organic sulfur transformations [1–5]. Additionally, they are an important group of compounds due to their unique role in medicine, food, pharmaceutical and aroma industries [6–8]. Together, these characteristics have led to considerable interest in thiolic compounds such as cysteine (CySH) and glutathione (GSH) which have critical functions in physiological processes in a variety of living organisms, ranging from microbes to humans [9–12]. As a result, considerable effort has gone into developing accurate and sensitive methodologies to extract and measure thiols in a variety of sample matrices [13–18] or in the determination of several thiol drugs [9,19]. However, less attention has been given to other low-molecular-weight hydrophilic thiols, such as 3-mercaptopropionic acid ($HSCH_2CH_2CO_2H$; 3-MPA), which are found in significant concentrations in natural aquatic environments [20–23].

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In this study we focused on developing a sensitive and accurate method to measure 3-MPA. This thiol is a major natural organic sulfur compound (thiol) produced during microbial decomposition of sulfur-containing amino acids like methionine and homocysteine [1,20,21,24,25]. In natural environments, 3-MPA can also be formed during microbial decomposition of the osmolyte dimethylsulfoniopropionate (DMSP), an important carbon and sulfur source to marine bacterial communities. 3-MPA is produced from DMSP through two successive demethylations [20,26]. Demethylation of DMSP is an alternative pathway to the so-called cleavage pathway which produces dimethyl sulfide (DMS), a sulfur gas with a potential role in climate [27]. Production of 3-MPA has been demonstrated to occur in aerobic bacterial cultures and anoxic sediments [1,20,25,28,29] from 3-methiolpropionate (MMPA), which is the first product during the double demethylation of DMSP [26,30]. Since 3-MPA is a central compound in natural organic sulfur geochemistry [4,25,28], its determination remains important in biogeochemical and environmental studies. However, only few methods have been developed to quantify it in different matrices, which limits our knowledge of 3-MPA transformations and interactions in natural environments. Previously described methods for thiol analysis in biological samples were based on *o*-phthalaldehyde (OPA) for pre- or post-column derivatizations in HPLC, in which thiols react to form fluorescent derivatives [e.g. 31–33]. While sensitive, the OPA derivatization method has several disadvantages, namely the rapid degradation of derivatives. Also, since thiols are highly susceptible to oxidation with molecular oxygen, samples must be processed immediately [2,20], and this poses practical limitations with the OPA method because the derivatives are unstable. Recently, a new microchip capillary electrophoresis (CE)-based method has been developed for the determination of thiols (e.g. 2-MPA) in cosmetics (depilatory cream and wave lotions) after their derivatization with the fluorogenic reagent ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F) [17]. This work reported that microchip CE analysis requires small amounts of sample and reagents and that separation times can be reduced compared to conventional methods. Nevertheless, the detection limit is relatively high (2 μmol L^{-1}) which becomes limiting to quantify 3-MPA in natural environments with typical concentrations ranging from nmol L^{-1} to μmol L^{-1} levels. In fact, previous analytical methods for measuring 3-MPA have already established a limit of detection (LOD) at ultratrace levels for the thiol determinations [2,25,32].

The methodology used in the present study was based on the Newton and Fahey [34] derivatization method with monobromobimane (MBBr) followed by high-performance liquid chromatography (HPLC) analysis, a method previously used for the determination of biothiols (CySH and GSH) and thiol drugs (cysteamine, penicillamine and mercaptopropionylglycine). The method was optimized for a quick sample analysis, long-term storage of derivatized samples and allowing the measurement of 3-MPA at nmol L^{-1} concentrations.

2. Materials and methods

2.1. Chemicals and reagents

The 3-mercaptopropionic acid (3-MPA) used had a purity of ≥99%. The remaining reagents, 6 mmol L^{-1} dithiothreitol (DTT, Fluka) and 0.2 mol L^{-1} 2-(cyclohexylamino) ethanesulfonic acid (CHES) were prepared in pure HPLC grade water. The solution of 1 mol L^{-1} Tris(hydroxymethyl)-aminomethane hydrochoride (Tris-HCl) was prepared from 6 mol L^{-1} hydrochloric acid (HCl) and Tris base (Duchefa Biochemie) ultrapure grade ≥99.9%, were prepared in pure HPLC grade water. The derivatizing reagent solution, 30 mmol L^{-1} MBBr, was prepared in 100% of HPLC grade methanol. Pure HPLC grade water, analytical grade acetic acid, 3-MPA, HCl, MBBr, CHES and methanol were obtained from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Method development

2.2.1. HPLC conditions

Chromatographic analyses were performed with a Shimadzu LC-20AD high-performance liquid chromatograph (HPLC, Analytical and Measuring Instruments Division, Kyoto, Japan). This HPLC was equipped with two LC-10AD pumps, SIL-20ACHT automatic sampler with an injection-volume setting range of 0.1–100 μL and a CTO-10ASvp column oven. Detection was performed with a Shimadzu high pressure RF-20A XS fluorescence detector at excitation of λ 380 nm and emission of λ 480 nm. The chromatographic separation of the compounds was achieved with a *mediterranea* sea_{18} 5 μm (25 cm × 0.46 cm) column (Teknokroma, Spain) operating at 20 °C. The eluent consisted of two methanol–aqueous (pure water) mobile phases (mobile phase A was 2:98 (v/v) and mobile phase B was 90:10 (v/v)), at a flow rate of 0.9 mL min^{-1}, with a linear gradient as follows: 1 min 7% B, 5 min 30% B, 35 min 60% B, 35.01 min 7% B (mobile phase B as default), maintaining these conditions for 10 min and returning to the initial conditions. Data were analyzed using LCsolution Version 1.25 Software (Shimadzu, Japan).

2.2.2. Method validation

Our analytical method was validated by a set of verification parameters such as linearity, accuracy, precision, recovery, sensitivity and stability, in order to corroborate the reliability of obtained results. In addition, measurements of the analyte were also performed in estuarine waters [35].

The linearity of the method was tested using an external standard calibration with different concentration levels of 3-MPA standard solutions. A total of six and five standards were used for high (1, 5, 10, 15, 20 and 25 μmol L^{-1}) and low (0.005, 0.01, 0.05, 0.1 and 0.2 μmol L^{-1}) concentration calibration curves, respectively. Each derivatized standard was injected at a 10 μL volume into the HPLC in duplicate, in order to account for the inherent HPLC variability between different runs. The calibration curve, the relationship between the known concentration and detector response (peak area) of the analyte, was determined by a linear regression.

Accuracy (Bias %) was evaluated by determination of 3-MPA recoveries from spiked estuarine water samples (see below) and spiked HPLC grade water. The accuracy with different high and low concentrations was estimated based on Causon [36]: Bias (%) = [(measured value − true value)/true value] × 100. Recovery of 3-MPA was calculated by comparing the detector response of added 3-MPA at four fortification levels (1 μmol L^{-1}, 5 μmol L^{-1}, 10 μmol L^{-1} and 20 μmol L^{-1}) to HPLC grade water (standard solutions) and to environmental estuarine waters with different salinities (0.17 and 35.85 ppt) according to Cassiano et al. [37]. To calculate the recovery values, the contribution of 3-MPA in the unspiked "blank" sample was subtracted from the corresponding value obtained in each spiked sample.

Precision of the method was evaluated through repeatability of intra- and inter-day assays. The former was conducted by measuring five replicates of different concentrations (1, 10 and 25 μmol L^{-1}) in the same day and the latter by repeating assays on six different days with two replicates at each of six different fortification levels (1–25 μmol L^{-1}).

This parameter is expressed as the coefficient of variation (CV%): CV (%) = (standard deviation/mean) × 100. Based on Causon [36], both precision and accuracy measures should be within ±15% for a reliable method.

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