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Ratiometric quantitation of thiol metabolites using non-isotopic mass tags

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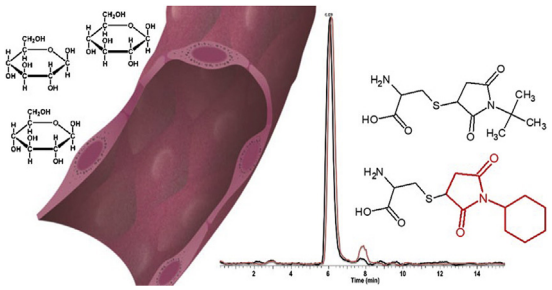
GRAPHICAL ABSTRACT



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ABSTRACT

Ratiometric quantitation is used in mass spectrometry to account for variations in ionization efficiencies due to heterogenous sample matrixes. Isotopes are most commonly used to achieve ratiometric quantitation because of their ability to co-elute chromatographically with each other and to have similar ionization efficiencies. In the work presented here, a new non-isotopic quantitative tagging approach is presented which allows chromatographic co-elution and similar ionization efficiencies. Using two variations of maleimide tags, *t*-butyl and cyclohexyl maleimide, thiols are quantified with a high degree of linearity up to five-fold concentration differences. Because these two tags have similar hydrophobcities, they elute simultaneously which allows them to be used for ratiometric quantitation. Beyond the five-fold linear range, signal compression is observed. This technique was able to quantify thiol changes in both *in vitro* pharmacological treatments as well as *in vivo* diabetic tissue.

1. Introduction

Quantitation in electrospray ionization mass spectrometry (ESI-MS) is complicated by competing ionization, adduct formation and overall variability in the ESI process [1,2]. MS most often uses stable isotope dilution or stable isotope chemical tagging to account for signal variability [3–9]. The use of stable isotopes for ratiometric quantitation is possible because the heavy and light isotopes create a mass shift detectable by the MS but do not create differential ionization [10]. For optimal benefit, the heavy isotopes need to be ionized at the same time as the light isotopes. For direct infusion, co-ionization is not an issue, but for condensed phase separations, the need for co-elution may be a complicating factor. While most stable isotopes (^{13}C, ^{15}N, ^{18}O), do not affect liquid phase separations (LC), deuterium does cause a chromatographic shift under reverse

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phase (RP) conditions and is therefore often deemed undesirable despite its lower cost [1,11].

Use of stable isotopes to quantitate thiol metabolites has previously been successfully undertaken [1,11]. Thiol metabolism is of great interest to many disease states (e.g. diabetes) given the involvement in oxidative stress [12,13]. Glutathione (GSH) is an endogenous antioxidant thiol peptide formed through the sulfur metabolic pathway (Fig. 1) [14,15]. The sulfur metabolic pathway involves homocysteine, cysteine, γ-glutamyl-cystine (Glu-Cys), Cysteine-glycine (Cys-Gly), and GSH [15]. Detection of this pathway has often used electrochemistry [16,17], fluorescence [18] or MS coupled to RPLC detection [11]. For MS analysis, chemical tagging of the thiols is has been used due the ion-suppressive effects inherent in thiols. Tagging of thiols for MS detection uses either a maleimide based tag or iodoacetate group. In comparing these tags, maleimide kinetics are generally quicker (5–30 min reaction vs. 12 h for

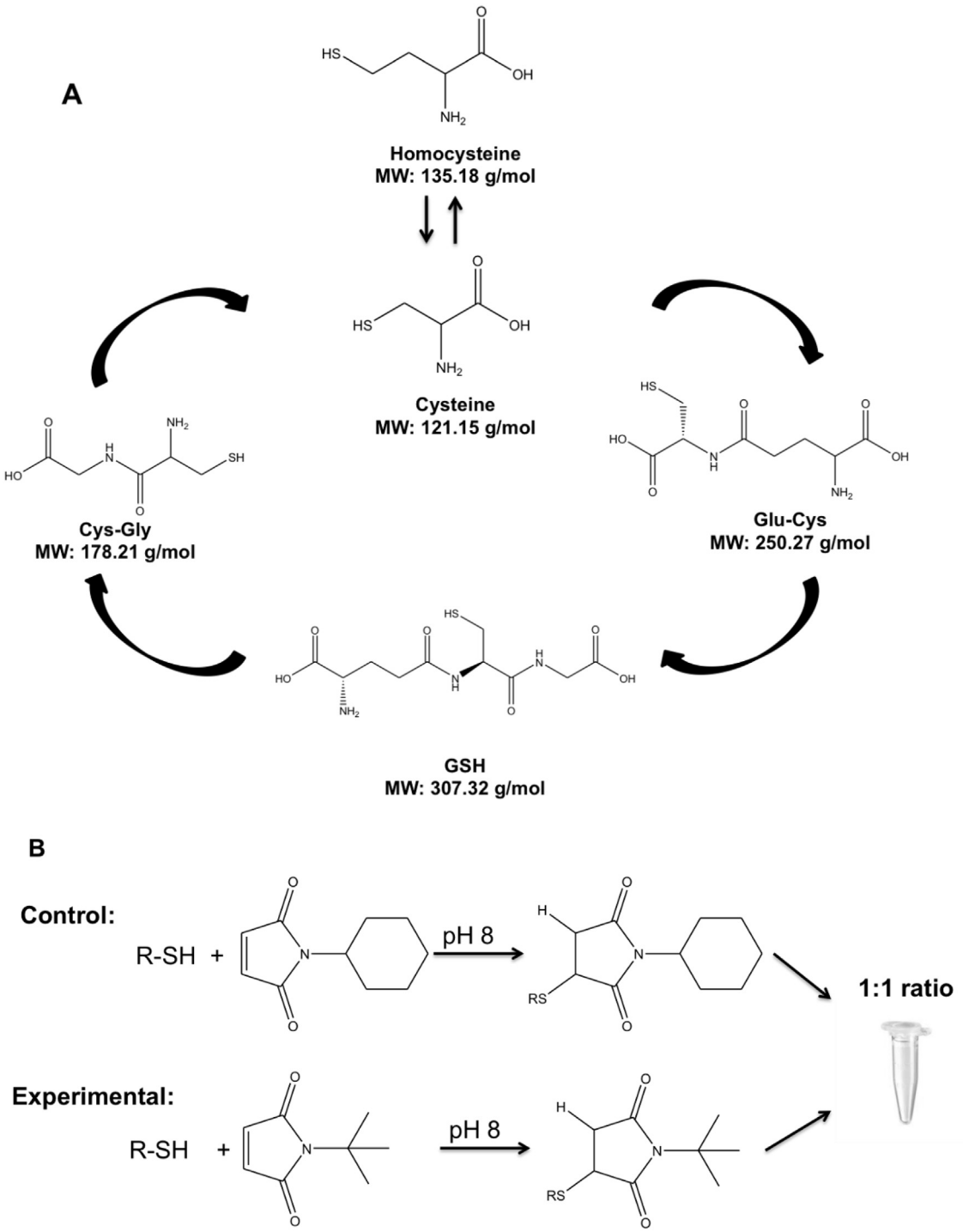

Fig. 1. Sulfur Metabolism and Tagging Regime. A. Structure and molecular weight of thiols that participate in the sulfur metabolic pathway. B. Alkyl maleimides are reacted with reduced thiol in 80% acetonitrile solution with 5 mM ammonium formate at pH 8. Tagged thiols were combined at 1:1 ratio and analyzed by HILIC-MS/MS. The mass tags yield differing molecular weights but identical retention times by HILIC separation.

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