Quantification of methionine and selenomethionine in biological samples using multiple reaction monitoring high performance liquid chromatography tandem mass spectrometry (MRM-HPLC-MS/MS)

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Abstract:

Quantification of selenated amino-acids currently relies on methods employing inductively coupled plasma mass spectrometry (ICP-MS). Although very accurate, these methods do not allow the simultaneous determination of standard amino-acids, hampering the comparison of the content of selenated versus non-selenated species such as methionine (Met) and selenomethionine (SeMet). This paper reports two approaches for the simultaneous quantification of Met and SeMet. In the first approach, standard enzymatic hydrolysis employing Protease XIV was applied for the preparation of samples. The second approach utilized methanesulfonic acid (MA) for the hydrolysis of samples, either in a reflux system or in a microwave oven, followed by derivatization with diethyl ethoxymethylenemalonate. The prepared samples were then analyzed by multiple reaction monitoring high performance liquid chromatography tandem mass spectrometry (MRM-HPLC-MS/MS). Both approaches provided platforms for the accurate determination of selenium/sulfur substitution rate in Met. Moreover the second approach also provided accurate simultaneous quantification of Met and SeMet with a low limit of detection, low limit of quantification and wide linearity range, comparable to the commonly used gas chromatography mass spectrometry (GC-MS) method or ICP-MS. The novel method was validated using certified reference material in conjunction with the GC-MS reference method.

Key words: methionine, selenomethionine, Protease XIV, methanesulfonic acid, diethyl ethoxymethylenemalonate, liquid chromatography mass spectrometry.

Abbreviations:

Met: methionine; SeMet: selenomethionine; GC-MS: gas chromatography mass spectrometry, HPLC-ICP-MS: high performance liquid chromatography inductively coupled plasma mass spectrometry; HPLC-MS: high performance liquid chromatography mass spectrometry;