Journal of Chromatography B, 955–956 (2014) 34–41

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Matrix influence on derivatization and ionization processes during selenoamino acid liquid chromatography electrospray ionization mass spectrometric analysis

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ARTICLE INFO

Article history:
Received 5 October 2013
Received in revised form 11 January 2014
Accepted 8 February 2014
Available online 18 February 2014

Keywords:
Amino acids
Derivatization
Matrix effects
LC/ESI/MS

ABSTRACT

Considering the importance of derivatization in LC/ESI/MS analysis, the objective of this work was to develop a method for evaluation of matrix effect that would discriminate between matrix effect due to the derivatization reaction yield and from the ESI. Four derivatization reagents (TAHS, DEEMM, DNS, FMOC-Cl) were studied with respect to matrix effects using two selenoamino acids and onion matrix as model system. A novel method for assessing matrix effects of LC/ESI/MS analyses involving derivatization is proposed, named herein post-derivatization spiking, that allows evaluating effect of matrix on ESI ionization without derivatization reaction yield contribution. The proposed post-derivatization spiking method allowed to demonstrate that the reason of reduced analytical signal can be signal suppression in ESI (as in case of DNS derivatives with matrix effects 38–99%), alteration of derivatization reaction yield (TAHS, matrix effects 92–113%, but reaction yields 20–50%) or both (FMOC-Cl, matrix effects 28–88% and reaction yields 50–70%). In case of DEEMM derivatives, matrix reduces reaction yield but enhances ESI/MS signal.

A method for matrix effect evaluation was developed. It was also confirmed that matrix effects can be reduced by dilution.

1. Introduction

Amino acids are analyzed for various reasons in all types of biological samples ranging from human bodily fluids and tissues to various foods. For example, in clinical biochemistry, changes in amino acid concentrations in human serum can be correlated to certain diseases [1]. Similarly, selenoamino acids such as Se-methylselenocysteine (Se-MeSeCys) and selenomethionine (SeMet) are under interest in clinical studies [2] (Fig. 1) and since they have anticarcinogenic properties, these are determined in various foods, such as garlic [3,4]. In all mentioned cases, very low concentrations of amino acids are targeted in complex matrices.

Due to the constant pursuit for more sensitive analysis, liquid chromatography mass spectrometry (LC/MS) with electrospray ionization (ESI) has become one of the most popular analysis techniques for many analytes, including selenoamino acids [5]. In addition to low limits of detection, it enables to confirm the identity of analytes and identify unknown compounds [6].

When analyzing amino acids, traditionally, derivatization has been applied in order to enhance the sensitivity of ultraviolet–visible detection (UV–Vis) and also to improve the chromatographic separation [7]. Employing derivatization for LC/ESI/MS is a newer approach, but the aims are similar: to increase detection sensitivity and selectivity by means of MS/MS technique, improve chromatographic retention or peak shape, eliminate carryover, facilitate sample cleanup, and to form a stable derivative for unstable analytes [8].

For the LC/ESI/MS analysis of amino acids, "classical" derivatization reagents are often used, for example dansyl chloride (DNS) [9], 9-fluorenylmethyl chloroformate (FMOC-Cl), and diethyl ethoxymethylenemalonate (DEEMM) [10], which have been designed for UV absorbance or/and fluorescence detectors. In recent years, there has been a rapid growth in designing and developing amino acid derivatization reagents that are specially meant for LC/ESI/MS applications for lowering detection limits, e.g. 3-aminopyridyl-*N*-hydroxysuccinimidyl carbamate (APDS) [11], *N*-hydroxysuccinimide ester of *N*-alkylnicotinic acid (C_n-NA-NHS) [12] and p-*N,N,N*-trimethylammonioanilyl *N*′-hydroxysuccinimidyl carbamate iodide (TAHS) [13]. However, these reagents are not commercially available and in

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http://dx.doi.org/10.1016/j.jchromb.2014.02.016
1570-0232/

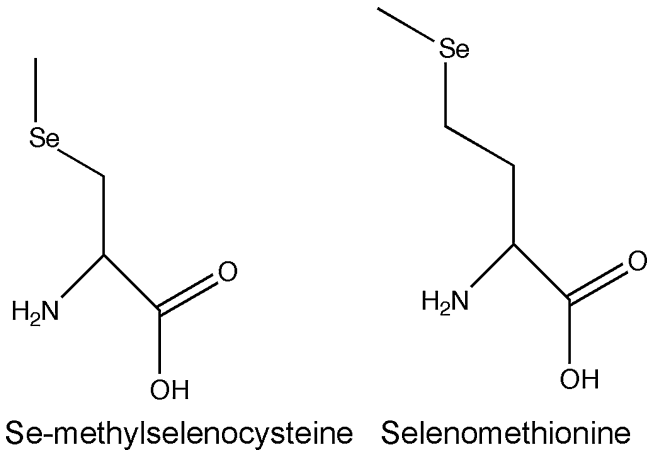

Fig. 1. Structures of Se-methylselenocysteine and selenomethionine.

thoroughly optimized conditions, commercially available derivatization reagents can provide similar sensitivity [10].

When combination of derivatization and LC/ESI/MS analysis is applied on real samples, multiple aspects are to be considered. Firstly, in the ESI source, the efficiency of generating gas phase ions from a compound in solution depends mainly on the properties of the compound [14]. Importantly, compounds other than analyte in the ESI source can have a considerable effect on the ionization of the analyte. If the compounds causing suppression or enhancement of an analyte signal originate from the sample matrix, the effect is called the matrix effect [15,16]. In general, matrix effects may be caused by the compounds of current or previous injections, either as late-eluting peaks (bands) or as impurities depositing on the internal surfaces of ESI source [17]. King et al. analyzed biological samples and concluded that the ionization suppression is most likely resulted by the high concentration of nonvolatile compounds present in the spray concurrently with the analyte [18].

The matrix effect (*ME%*) can be quantitatively expressed by Eq. (1), where A_{matrix} and $A_{standard}$ are peak areas of the equal amount of analyte, respectively, in presence and in absence of possibly interfering compounds. The *ME%* value of 100% indicates that the ionization of an analyte is not affected by other compounds present in the ESI source. *ME%* values below and above 100% indicate ionization suppression and enhancement, respectively [19].

$$ME\% = \frac{A_{matrix}}{A_{standard}} \times 100\% \quad (1)$$

This technique is also called post-extraction spiking: analytical signal of blank sample extract spiked with the analyte (A_{matrix}) is compared with the signal of the equal amount of analyte in pure solvent ($A_{standard}$) [17].

Another technique for evaluation of matrix effects is post-column infusion, a method where using a syringe pump and a tee-piece, a continuous flow of an analyte is mixed with the chromatographic effluent of sample blank analysis [17]. However, this approach does not give quantitative information about the matrix effect [20].

For certain types of analytes, such as amino acids, a blank sample is sometimes not available. For example, in case of honey or blood plasma analysis, amino acid free sample does not exist and it is quite difficult to get an estimation of matrix effect. Spiking technique can sometimes help, but there are problems related to this approach also [19]. On the other hand, for selenoamino acids, blank matrices are available, since selenium concentration in plants depends on the soil it is grown in. Therefore, onions grown in regions such as Middle and North Europe do not contain selenoamino acids [21]. These samples can be used for matrix effect estimation of methods analyzing selenoamino acids in onion samples.

With methods that contain derivatization step, the matrix effect is often studied as in case of a regular analytical method. For example, for β-alanine propyl chloroformate derivative, matrix effects have been evaluated with continuously infusing purified derivative with LC-effluent through tee-piece after analytical column. For matrix effect assessment, signal of the β-alanine propyl chloroformate derivative was monitored [22]. This approach is somewhat limited since in order to get information for all amino acids, derivatives purified from derivatization reaction byproducts and buffer components must be obtained, and even then quantitative information about the matrix effects is not obtained.

Derivatization step adds complexity to the quantitative assessment of matrix effects since derivatization mixture contains additional components such as nonvolatile borate buffer [1], which has been shown to cause signal suppression in the ESI source [23]. Moreover, previously described approaches of the matrix effect assessments do not take into account the reaction yield of the derivatization (it is assumed to be the same for the standard solutions and the matrix). With derivatization reactions, there is always a possibility that part of the analytes remain unreacted. This is well illustrated by the fact that there are many publications dedicated to the optimization of derivatization reactions [24–26]. Therefore, while investigating matrix effects in the ESI source, there is a risk that poor derivatization reaction yield may be regarded as matrix effect.

Considering the importance of derivatization in LC/ESI/MS analysis, evaluation of its matrix effects deserves in-depth analysis. The objective of this work is to develop a method for the evaluation of matrix effect in complex matrices. The method assesses the two matrix effect components that are 1) due to the derivatization reaction yield and 2) from the ESI-ionization.

2. Materials and methods

2.1. Chemicals

HPLC-grade methanol and acetonitrile were obtained from Sigma-Aldrich. Derivatization reagents diethyl ethoxymethylenemalonate (DEEMM) and dansyl chloride (DNS) were purchased from Fluka and 9-fluorenylmethyl chloroformate (FMOC-Cl) was purchased from Aldrich. Se-MeSeCys was kindly donated by LGC Limited (United Kingdom) and SeMet was purchased from Sigma. Other chemicals: sodium hydroxide (Chemapol); acetic acid (Lach-Ner); sodium dihydrogensulfate (Merck); hydrochloric acid, boric acid, and ammonium hydroxide were from Reakhim; formic acid and ammonium acetate from Fluka. All reagents were of analytical grade unless otherwise stated.

All aqueous solutions were prepared with ultrapure water purified by Millipore Milli-Q Advantage A10 (Millipore).

Onion samples were obtained from the local market and the stated country of origin was the Netherlands.

2.2. Preparation of standard solutions

Se-MeSeCys stock solution (0.55 mg g^{-1}) was prepared by dissolving the amino acid in 0.1 M hydrochloric acid with 30% MeOH. Stock solution of SeMet (0.4 mg g^{-1}) was prepared in 0.5% 2-mercaptoethanol aqueous solution. Stock solutions were prepared once and stored at −20 °C. All dilutions (0.5–12 μg g^{-1}) were made with 0.004% aqueous solution of 2-mercaptoethanol to avoid oxidation of SeMet [27]. Working standard solutions were prepared daily.