Analytical Biochemistry 539 (2017) 158–161

Contents lists available at ScienceDirect

Analytical Biochemistry

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

Quantification of sulphur amino acids by ultra-high performance liquid chromatography in aquatic invertebrates

Jennifer C. Thera[a,*], Karen A. Kidd[a,b], M. Elaine Dodge-Lynch[c], Robert F. Bertolo[c]

[a] Biology Department and Canadian Rivers Institute, University of New Brunswick, 100 Tucker Park Road, Saint John, NB, E2L 4L5, Canada
[b] Department of Biology & School of Geography and Earth Sciences, McMaster University, 1280 Main Street W., Hamilton, ON, L8S 4K1, Canada
[c] Memorial University of Newfoundland, 230 Elizabeth Ave, St. John's, NL, A1B 3X9, Canada

ARTICLE INFO

Keywords:
Ultra-high performance liquid chromatography
Invertebrate
Cysteic acid
Methionine sulfone

ABSTRACT

We examined the performance of an ultra-high performance liquid chromatography method to quantify protein-bound sulphur amino acids in zooplankton. Both cysteic acid and methionine sulfone were linear from 5 to 250 pmol ($r^2 = 0.99$), with a method detection limit of 13 pmol and 9 pmol, respectively. Although there was no matrix effect on linearity, adjacent peaks and co-eluting noise from the invertebrate proteins increased the detection limits when compared to common standards. Overall, performance characteristics were reproducible and accurate, and provide a means for quantifying sulphur amino acids in aquatic invertebrates, an understudied group.

Introduction

Sulphur amino acids, methionine and cysteine, play a fundamental role in protein synthesis, structure, and function [1] and in the storage of contaminants such as the neurotoxin methylmercury (MeHg) [2]. MeHg binds to sulphur amino acids during uptake, storage, and elimination in aquatic organisms including phytoplankton, bacteria, and fish [3–5] and is almost exclusively present as MeHg-cysteine in fish muscle proteins [6,7].

Accurate quantification of protein-bound sulphur amino acids is therefore desirable, but current methods present several challenges. Under classic hydrolysis conditions (6 N HCl, 110 °C, 24 h [8]), methionine and cysteine are destroyed to various degrees; consequently, they are usually oxidized to methionine sulfone (MSO) and cysteic acid (CYA), respectively, using performic acid (PFA) prior to acid hydrolysis [9]. Most studies reporting amino acid compositions of tissues are often lacking data for cyst(e)ine and methionine because of the extra time for this oxidation process, loss of other amino acids during PFA oxidation, and low levels of sulphur amino acids present [8,10,11]. Such data are particularly lacking in invertebrate samples, which have a different hydrolysate matrix than mammalian or plant tissues.

The newer ultra-high performance liquid chromatography (UPLC) systems increase speed, performance, and sensitivity for amino acid analyses when compared with previous technologies [12], thereby requiring less sample mass which is a particular advantage for some environmental samples. Although PFA oxidation with HPLC is a well-established method, to our knowledge the current work represents the first time a UPLC was used to validate the detection of sulphur amino acids.

Samples

Zooplankton are microcrustaceans living in the water column of diverse aquatic habitats and form a key food resource for larval fish. Zooplankton (> 153 μm, bulk - Branchiopoda & Maxillopoda classes) from Kejimkujik National Park, Nova Scotia, Canada was selected as the invertebrate taxa for this study because it was possible to collect a large mass relatively easily. The bulk sample was collected in 2013 by towing a Wisconsin net in the pelagic zone of a lake and kept on ice until frozen. Prior to analysis, the sample was lyophilized, homogenized, and stored at –20 °C, consistent with standard procedures [8].

Chromatography

UPLC analysis was performed using a Waters Acquity system with a binary solvent manager, autosampler, column heater, and fluorescence detector (Milford, MA, USA) set up according to the Waters UPLC system guide [13]. Briefly, all chromatographic separations were performed with an in-line filter and a Waters AccQ·Tag$_{ultra}$ reverse-phase column (2.1 × 100 mm, 1.7 μm) and fluorescence detector with excitation and emission wavelengths of 266 and 473 nm, respectively. The column heater was set at 55 °C and the mobile phase flow rate was

* Corresponding author.
E-mail address: JenniferThera@gmail.com (J.C. Thera).

http://dx.doi.org/10.1016/j.ab.2017.10.022
Received 6 May 2017; Received in revised form 13 October 2017; Accepted 25 October 2017
Available online 26 October 2017
0003-2697/

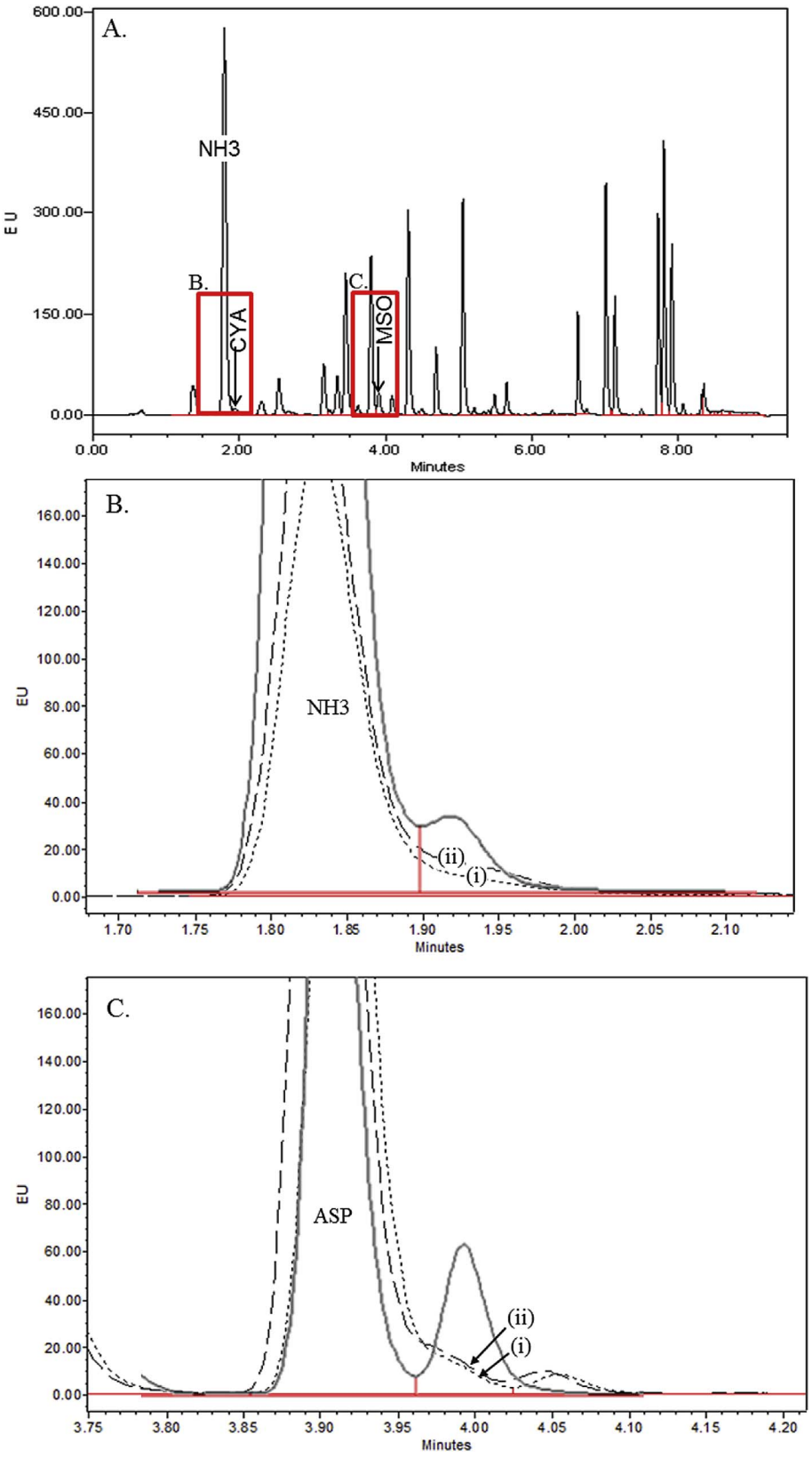

Fig. 1. Ultra-high performance liquid chromatography with fluorescence detection chromatogram of amino acids from (A) a zooplankton hydrolysate with performic acid (PFA) oxidation showing the location of ammonia (NH_3), cysteic acid (CYA), and methionine sulfone (MSO) peaks. Chromatograms of unoxidized zooplankton hydrolysates spiked with (B) CYA and (C) MSO standards of (i) 0 and (ii) 12.5 pmol/μL.

maintained at 0.7 mL/min. Eluent A was 5% (v/v) AccQ·Tag$_{ultra}$ eluent A concentrate: 95% (v/v) Milli-Q ultrapure water and eluent B was 98% (v/v) acetronitrile: 2% (v/v) formic acid. The gradient profile was 0–0.54 min (99.9% A), 5.74 min (90.0% A), 7.74 min (78.8% A), 8.04 min (40.4% A), 8.05 min (10.0% A) 8.73–10.00 min (99.9% A). Injection volume was 1 μL. Empower 3 (Waters) chromatography software was used for data acquisition.