Journal of Chromatography A, 1458 (2016) 145–149

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Short communication

Optimization of a single phase method for lipid extraction from milk

Zhiqian Liu [a,*], Simone Rochfort [a,b], Benjamin G. Cocks [a,b]

[a] Biosciences Research, Department of Economic Development, Jobs, Transport and Resources, AgriBio, 5 Ring Road, Bundoora, Victoria 3083, Australia
[b] School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3083, Australia

ARTICLE INFO

Article history:
Received 16 March 2016
Received in revised form 19 May 2016
Accepted 16 June 2016
Available online 17 June 2016

Keywords:
Milk
Lipid
One-phase extraction
Liquid chromatography–mass spectrometry

ABSTRACT

For LC–MS-based lipidomic analysis of milk, total lipid extraction from raw milk is generally conducted with Folch or Bligh and Dyer methods; both methods are based on two-phase partition of lipids, and thus time-consuming. In this work, three solvent systems for one-phase extraction of milk lipids were compared with the standard Folch method. Two of the solvent systems (butanol/methanol, 3:1 and 1:1) previously tested for lipid extraction from plasma were found to provide adequate extraction for polar lipids, but incomplete extraction for triglycerides, especially highly lipophilic species. By contrast, our newly designed solvent mix composed of butanol, methanol and chloroform (at a 3:5:4 ratio) provided similar extraction efficiency for triglycerides and higher yield for some of the phospholipids, as compared to the Folch method. This new one-phase extraction method is very simple yet comprehensive and thus suitable for high throughput lipid analysis of milk samples.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Fatty acid composition and the isomeric form of glycerolipids in milk determines the absorption and metabolism of milk fat by the human body, and has a profound influence on the property and quality of dairy products [1,2]. Liquid chromatography coupled to mass spectrometry (LC–MS) is the prevalent technique for the characterization of lipids at the molecular species level. Generally, prior to LC–MS analysis, total lipid is extracted from milk samples using Folch or Bligh and Dyer methods [3,4]. Both methods are based on the use of a chloroform-methanol-water mix to create a two-phase system and the partition of the lipid fraction in the organic (chloroform) phase. At least two cycles of extraction are generally required to provide adequate recovery. In addition, removing chloroform and reconstituting the extracted lipids in a different solvent system is frequently needed before LC–MS analysis. Consequently, the entire lipid extraction procedure is tedious and low throughput.

Simpler lipid extraction procedures have been tested. A one-phase extraction method was first tested with plasma samples using chloroform/methanol (2:1) added to plasma at a 20:1 ratio [5]. After extraction, this method required the removal of the extraction solvent and reconstitution of the sample in a different solvent system to give satisfactory chromatographic performance. Recently, Alshehry et al. [6] tested two more one-phase solvent systems (butanol/methanol, 3:1 and 1:1) for extracting plasma lipids and found that butanol/methanol (1:1) mixed with plasma (at a 10:1 ratio) resulted in efficient extraction of all major lipid classes and the extracted sample could be analysed directly by LC–MS. This prompted us to search for a one-phase lipid extraction method suitable for milk samples. We report here the comparison of the two previously described solvents systems (*i.e.* butanol/methanol at 1:1 and 3:1 ratios) and a new one-phase solvent mix (butanol/methanol/chloroform, 3:5:4) with the standard Folch method in lipid extraction efficiency from raw milk.

2. Materials and methods

2.1. Chemicals and reagents

Three triglyceride (TAG) standards tricaprin (TAG 30:0), tripentadecanoin (TAG 45:0) and tristearin (TAG 54:0), and five polar lipid standards phosphatidylcholine (PC 32:0), phosphatidylethanolamine (PE 32:0), phosphatidylserine (PS 36:4), phosphatidylinositol (PI 34:2) and sphingomyelin (SM 36:1) were purchased from Sigma-Aldrich. Solvents used for lipid extraction and mobile phase prepration were of chromatographic grade and were from Merck (methanol, butanol and acetonitrile) and Sigma-Aldrich (chloroform and isopropanol). Ammonium formate, used as mobile phase additive, was of analytical grade (Sigma-Aldrich).

2.2. Lipid extraction from milk

One bulked full cream milk sample obtained from the local market was used for method comparison. Three one-phase lipid

* Corresponding author.
E-mail address: Zhiqian.liu@ecodev.vic.gov.au (Z. Liu).

http://dx.doi.org/10.1016/j.chroma.2016.06.055
0021-9673/© 2016 Elsevier B.V. All rights reserved.

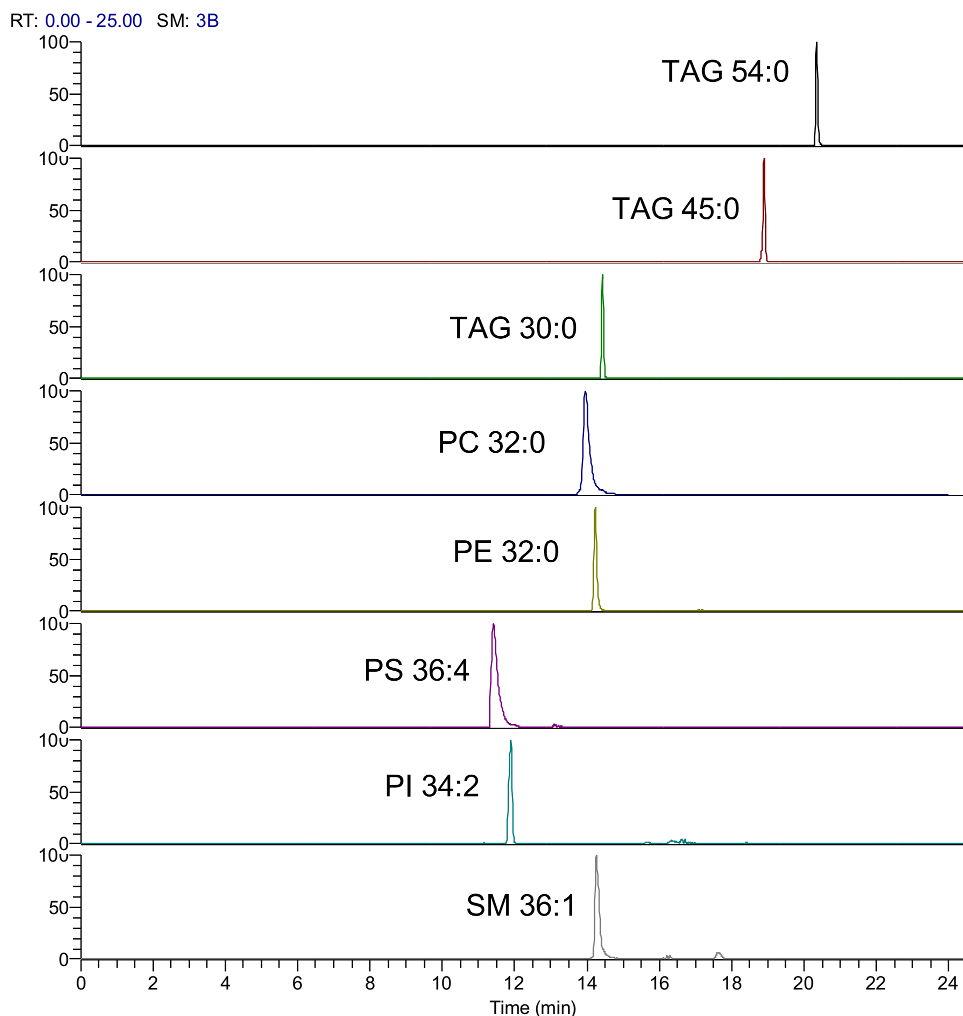

Fig. 1. LC–MS profile of spiked triglyceride and polar lipid standards after extraction by our new solvent mix butanol/methanol/chloroform (3:5:4).

extraction solvent systems (butanol/methanol at 3:1 and 1:1 ratios, and butanol/methanol/chloroform at a 3:5:4 ratio) were compared with the classic two-phase Folch method. For all the three one-phase extraction protocols, one mL of solvent mix was added to 100 μL of 3-fold diluted (in water) milk. The mixture was shaken by vortex for 20 s, sonicated for 20 min and then centrifuged for 15 min (15,000*g*). The supernatant was transferred to an injection vial and analysed directly by RP-LC–MS. In the case of the Folch method, milk lipid was extracted twice by chloroform/methanol (2:1). The combined organic phase was dried under a stream of nitrogen and the extract was reconstituted in isopropanol/chloroform (2:1) before LC–MS analysis.

The lipid extraction efficiency of the four methods was compared based on: 1) the percentage recovery of three TAG (TAG 30:0, TAG 45:0 and TAG 54:0) and five polar lipid (PC 32:0, PE 32:0, PS 36:4, PI 34:2 and SM 36:1) standards spiked to the milk sample; 2) the abundance (ion intensity) of representative TAG and polar lipid species of milk, immediately after extraction and after a 3-day storage at 12 °C. Both the recovery of spiked authentic standards and the extraction yield of milk native lipid species were determined with three replicates for each extraction method.

2.3. *Liquid chromatography–mass spectrometry*

Chromatographic separation of lipids was achieved using an Acquity UPLC HSS T3 column (100 × 2.1 mm, 1.8 μm, Waters) on an Agilent 1290 Infinity HPLC system. The column compartment was maintained at 50 °C and the auto-sampler at 12 °C. The mobile phase was composed of acetonitrile/water (60:40, v/v) containing 10 mM ammonium formate (A) and acetonitrile/isopropanol (10:90, v/v) containing 10 mM ammonium formate (B). The flow rate was 0.28 mL/min with a gradient elution of 20–100% B over 20 min. The injection volume was 5 μL. The lipid detection was by LTQ-Orbitrap mass spectrometer (Thermo Scientific) operated in electrospray ionization (ESI) positive or negative Fourier transform mode (resolution: 60,000 for both positive and negative modes). Identification of lipid species present in milk was performed using accurate mass of parent ions (±5 ppm) and MS^2 spectra as described previously [7,8]. Quantification of selected lipid species was based on peak area of parent ions extracted from the full scan spectrum using *Xcalibur* software (Thermo Scientific).

3. Results and discussion

The chromatographic performance of the eight spiked lipid standards extracted by the three one-phase solvent systems is comparable, all displaying consistent retention time and similar peak shape for the same lipid species. This indicates that the presence of chloroform in our new one-phase solvent mix (one third by volume) had no adverse effect. Indeed, without sample reconstitution (*i.e.* direct injection of the extract), this new one-phase solvent mix generated satisfactory peak shape for all the three TAG and five

Download English Version:

https://daneshyari.com/en/article/1198442

Download Persian Version:

https://daneshyari.com/article/1198442

Daneshyari.com