Journal of Chromatography B, 1041–1042 (2017) 70–76

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Automated milk fat extraction for the analyses of persistent organic pollutants

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ARTICLE INFO

Article history:
Received 14 July 2016
Received in revised form 28 October 2016
Accepted 3 December 2016
Available online 6 December 2016

Keywords:
POPs
Dioxins
Extraction
Automated
Acid hydrolysis

ABSTRACT

We have utilized an automated acid hydrolysis technology, followed by an abbreviated Soxhlet extraction technique to obtain fat from whole milk for the determination of persistent organic pollutants, namely polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls. The process simply involves (1) pouring the liquid milk into the hydrolysis beaker with reagents and standards, (2) drying the obtained fat on a filter paper and (3) obtaining pure fat via the modified Soxhlet extraction using 100 mL of hexane per sample. This technique is in contrast to traditional manually intense liquid-liquid extractions and avoids the preparatory step of freeze-drying the samples for pressurized liquid extractions. Along with these extraction improvements, analytical results closely agree between the methods, thus no quality has been compromised. The native spike (n = 12) and internal standard (n = 24) precision and accuracy results are within EPA Methods 1613 and 1668 limits. While the median (n = 6) Toxic Equivalency Quotient (TEQ) for polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans and the concentration of the marker polychlorinated biphenyls show a percent difference of 1% and 12%, respectively, compared to 315 previously analyzed milk samples at the same laboratory using liquid-liquid extraction. During our feasibility studies, both egg and fish tissue show substantial promise using this technique as well.

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1. Introduction

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (D/Fs) as well as polychlorinated biphenyls (PCBs) are a group of compounds known as persistent organic pollutants (POPs). These environmental contaminants are typically monitored by utilizing direct isotope dilution techniques as described in EPA Methods 1613 [1] and 1668 [2]. The D/Fs are commonly combined as one family, although they are two classes of the 12 initial POPs slated for elimination under the Stockholm Convention along with PCBs as a third class [3]. All three (D/Fs and PCBs) have toxicity effects, are lipophilic in nature, and have been detected in our food supply [4–19].

The major non-occupational exposure to D/Fs and PCBs comes through the diet by consuming animal fats in our foods. For example the Belgian PCB/Dioxin incident [4] while an isolated contamination of PCB oil to the feed supply, significantly contaminated the Belgian animal food supply. Additional examples of unintentional contaminations in feeds include ball clays in the United States in poultry [5] and catfish [6] feeds, bakery waste in Germany [7], and choline chloride that crossed borders in Europe [8]. Each of these cases illustrate where contaminated animal feeds have threatened our food supply. In the case of dairy animals exposed to contaminated feeds, these compounds are excreted into the milk and eventually appear in our daily diet [9].

Levels of POPs have been reported in milk from surveillance programs throughout the world. Tetra and penta-chlorinated dibenzofuran levels in milk fat from Iran [10] have recently been reported while additional cow milk surveillance programs have been and continue to be reported [11–13], including a study of seasonal variations in the U.K. [14]. Other dairy surveillance studies have also been completed including buffalo [15] goat [16] and sheep [17]. Some of the surveillance studies have resulted in unexpected findings leading to follow-up studies such as the citrus pulp incident [18] and Italian buffalo milk levels [19]. The U.S. Food and Drug Administration (U.S. FDA) completed a raw bovine milk surveillance study of about 320 samples during Fiscal Year 2013. A similar study was designed for the surveillance of “grocery-purchased”

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http://dx.doi.org/10.1016/j.jchromb.2016.12.005
1570-0232/Published by Elsevier B.V.

Table 1
Comparison of 315 Historical Milk Results Extracted by Liquid-Liquid Process to 6 Samples Extracted by the Automated Acid Hydrolysis Technique.

TEQ	n = 315 Sum of Dioxins (2005 WHO-PCDD/F-TEQ)	n = 6 Sum of Dioxins (2005 WHO-PCDD/F-TEQ)	n = 315 Sum of Dioxins and Dioxin-Like PCBs (2005 WHO-PCDD/F-TEQ)	n = 6 Sum of Dioxins and Dioxin-Like PCBs (2005 WHO-PCDD/F-TEQ)	n = 315 Sum of PCB28, PCB52, PCB101, PCB138, PCB 153 and PCB180	n = 6 Sum of PCB28, PCB52, PCB101, PCB138, PCB 153 and PCB181
Average	0.354	0.292	0.474	0.359	0.504	0.348
sd	0.207	0.069	0.245	0.058	0.454	0.041
Median	0.321	0.318	0.435	0.372	0.398	0.352

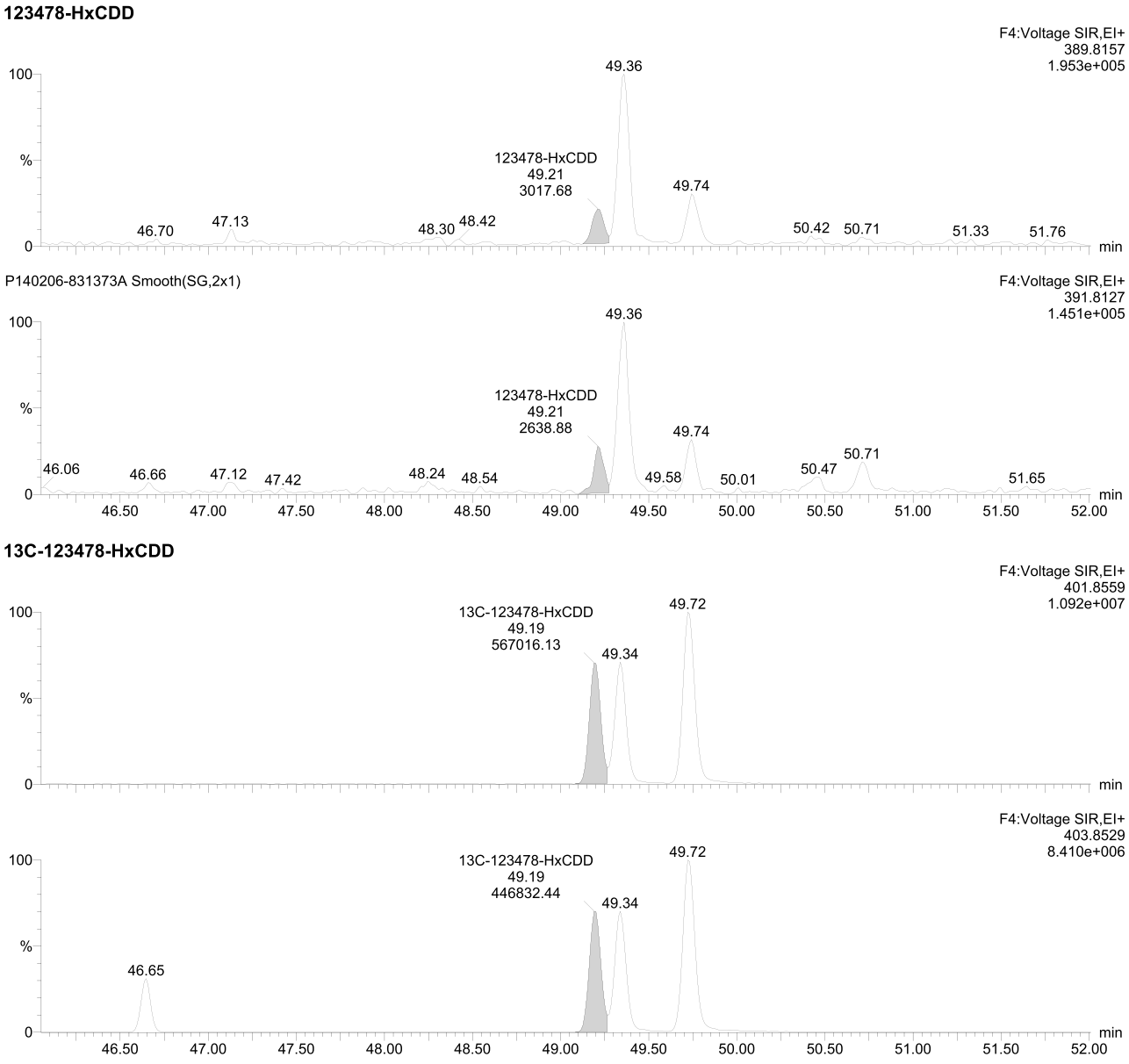

Fig. 1. The selected ion chromatograms shown in Fig. 1 represent typical responses for the hexachlorinated dibenzo-*p*-dioxin analytes obtained from milk extracts using the traditional liquid-liquid extraction. The first 2 chromatograms represent the M^+ and $(M+2)^+$ ion responses, masses 389.8 and 391.8, respectively, for the native or incurred levels analytes, while the following two chromatograms represent the M^+ and $(M+2)^+$ ion responses, masses 401.9 and 403.9, respectively, for the C-13 labeled internal standards, as described in EPA Method 1613 [1].

whole bovine milk (pasteurized) completed Fiscal Year 2014. This was followed by a new, "grocery-purchased" study for Fiscal Year 2016.

Several extraction techniques for the analyses of POPs in milk have been explored over the years. The goal for each is the same; first isolate the sample lipid material, followed by the isolation of the POPs from the lipid material. Recently concentrations have routinely been reported on a lipid weight basis. There are several methods for the determination of fat in milk that have been used, including Babcock [20], the Gerber Method [21], as well as other more recently developed and published techniques [22–24]. Most laboratories extract using some variation of liquid-liquid technique