Journal of Chromatography A, 1408 (2015) 169–177

Contents lists available at ScienceDirect

Journal of Chromatography A

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

# Ultra-trace quantification method for chlordecone in human fluids and tissues

Emmanuelle Bichon [a,*], Ingrid Guiffard [a], Anaïs Vénisseau [a], Philippe Marchand [a], Jean-Philippe Antignac [a,b], Bruno Le Bizec [a]

[a] LUNAM Université, Oniris, Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes F-44307, France
[b] INRA, Nantes F-44307, France

ARTICLE INFO

*Article history:*
Received 10 March 2015
Received in revised form 23 June 2015
Accepted 3 July 2015
Available online 8 July 2015

*Keywords:*
Chlordecone
Organochlorine pesticides
LC–MS/MS
Quantification
Ultra-trace
Multiresidue method

ABSTRACT

Chlordecone is an organochlorine pesticide (OCP) considered as a Persistent Organic Pollutant (POP) as it persists in the environment, bio-accumulates through the food web, causes adverse effects to human health and the environment and transports across international boundaries far from its sources. The atypical physico-chemical properties of chlordecone make its inclusion in classical analytical approaches non applicable. The aim of our work was to include chlordecone in a multi organochlorine residue method preventing any degradation during the analytical process and thus allowing quantification at ppt (ng $kg^{-1}$ or ng $L^{-1}$) levels for a wide range of OCPs in breast milk, human serum and adipose tissue. After GC–HRMS vs. MS/MS and EI vs. APCI comparisons, the major improvement in terms of sensitivity was found in decreasing the length and film thickness of the gas chromatography column. Thanks to a linear correlation between relative response and quantity of chlordecone injected, LC-(ESI-)-MS/MS was finally preferred. An acetonitrile based gradient optimized on a C30 coreshell HPLC column has led to reaching limits of quantification as low as 8 ng $L^{-1}$, 25 pg $mL^{-1}$ and 0.2 ng $g^{-1}$ fat for breast milk, serum and adipose tissue, respectively, allowing multiresidue OCP quantification at concentration levels compatible with biomonitoring purposes and pre-requisites.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

Chlordecone (also known as Kepone) is an organochlorine pesticide (OCP) which has been extensively used from 1972 to 1993, particularly in the French West Indies for protecting banana plantation against weevil attack [1]. Considered as Persistent Organic Pollutants (POPs) and thus included in the Stockholm convention [2], chlordecone is highly toxic and bioaccumulative and remains persistent in the environment. Its high stability and sorption on organic matter reveals a severe contamination of soils even twenty years after the ban [3–5]. A wide spread contamination of this OCP was identified in surface and wastewater [1] with bioaccumulation in fish [6] such as eels [7] or spotfish [8] and also in shrimps [9].

In 2012 and 2013, respectively, Dallaire and Boucher [10,11] focused their studies on the development of infants (7–18 months old) with pre- and postnatal exposure of chlordecone mainly based on concentrations of chlordecone in cord blood and breast milk (respectively 0.06–22.9 and 0.06–6.7 $\mu g\,L)^{-1}$. Conclusion of these studies suggests that exposure to environmental levels of chlordecone, OCP with well defined estrogen-like activity, is associated with persistent impairments in fine motor function during infancy. This effect was exclusively observed in boys. In adults, Multigner et al. [12] found in 2010 a significant increase in the risk of prostate cancer with increasing plasma chlordecone concentration. To limit chlordecone exposure, the French Food Safety Agency published a food exposure study on chlordecone in the French West Indies [13] since 2003. Root vegetables, meat, water, fish, fruits, shellfish, milk and eggs were at first controlled to establish maximum residues limits to protect the consumer from this chemical hazard. This study led to the recommendation of provisory maximal levels of between 50 and 100 $\mu g\,kg^{-1}$ for the priority list of food items, where root vegetables and chicken meat were identified as significant contributors to the chronic exposure of chlordecone.

To generate such external and internal exposure data for chlordecone, chemists developed various analytical strategies clearly characterized by different performances. As early as in the 1980s, Meijs [14] proposed a rapid and sensitive method to determine chlordecone in eels based on gas chromatography coupled to electron capture detection (GC–ECD) with a limit of quantification (LOQ) close to 10 $\mu g\,kg^{-1}$. Nowadays, ECD is still widely used

* Corresponding author. Tel.: +33 0240687880; fax: +33 0240687878.
*E-mail address:* emmanuelle.bichon@oniris-nantes.fr (E. Bichon).

http://dx.doi.org/10.1016/j.chroma.2015.07.013
0021-9673/© 2015 Elsevier B.V. All rights reserved.

because of its sensitivity, but mass spectrometry is recommended for confirmatory purposes, particularly at LOQ level [15].

Regarding sample preparation, analytical procedures are sticking to those used for POPs analysis in general, without major specificities. Extraction of the fatty content is performed by binary solvent mixtures, e.g. toluene/ethyl acetate (1:3, v/v), followed by petroleum ether/fuming sulfuric acid [9], hexane/acetone (85:15, v/v) [15], dichloromethane/acetone (50:50, v/v) [16] or by pressurized liquid extraction with hexane/dichloromethane/methanol (5:2:1, v/v/v) [17]. Clean-up is made either by liquid/liquid washing of the organic layer with sodium hydroxide, followed by sulfuric acid [15] or Florisil® purification [16]. Blood samples are predominantly treated according to the protocol published by Debier et al. [17] and originally dedicated to polychlorobiphenyls (PCB) analysis. It includes a C18 SPE purification where diethylether/hexane (85:15, v/v) is specifically used for chlordecone elution [12].

However, all these analytical procedures are mono-residue methods because of the particular physicochemical properties of chlordecone. Indeed, Chung [18] reviewed most critical points of organochlorine pesticide analysis in fatty food items and listed that chlordecone is known for not being resistant to sulfuric acid treatment typically used for fat removal, not being recovered under the high extraction temperatures sometimes used during the extraction process, and not being well separated from the other OCPs (isomers of BHC, endosulfan I, trans-nonachlor and alpha-chlordane) on conventional GC. Chung [18] also stated that chlordecone analysis is quite problematic and insensitive when GC is coupled to an MS analyser, even in negative chemical ionization mode. In the same way, Koesukwiwat et al. [19] does not encourage GC analysis for chlordecone measurements.

LC-(ESI-)-MS/MS could be an alternative and was already used to quantify chlordecone in animal tissues [20] and drinking water [1] by elution on a C18 functionalized silica column and single reaction monitoring based on the fragmentation of what they claimed to be the $[M+OH]^-$ precursor ion (507 > 427) [1,21]. Atmospheric pressure chemical ionization (APCI) was compared by Moriwaki and Hasegawa [21] to ESI without demonstrating superior capabilities in terms of sensitivity. These authors used a C30 functionalized silica column (5 μm porous particle size, 2 mm i.d. × 150 mm) which permitted to enhance the sensitivity down to 1 pg on-column (a methanol/water elution gradient was used).

The available methods in the literature are restricted either to multi OCP screening without chlordecone or focused on chlordecone only. The aim of our study was to develop a reliable analytical method compatible with the high-throughput ultra-trace quantification of chlordecone together with a wide range of other organochlorine pesticides in human adipose tissue, breast milk and serum. In this paper, different sample preparation strategies and spectrometric methods either based on LC or GC, and HRMS or MS/MS are discussed. A particular focus is made on chlordecone in the comments and illustrations; exhaustive results obtained for other OCPs are detailed in the appendices.

## 2. Material and methods

### 2.1. Reagents, solvents and phases

All the chemicals and solvents used are of high quality grade for trace analysis: acetonitrile was purchased from Carlo-Erba Reagents (Rodano, Italy), hexane, toluene, acetone, ethyl acetate, cyclohexane, ethanol, methanol, formic acid from Promochem (Molsheim, France), diethyl ether, dimethyl sulfoxide from Acros (Illkirch, France), Celite 545 from Serva (Paris, France), anhydrous sodium sulfate, dipotassium oxalate monohydrate from Merck (Darmstadt, Germany). Ultrapure water was obtained using a Nanopure system from Barnstead (Waltham, MA, USA). Saturated potassium oxalate solution at 35% was prepared by dissolving 35 g of dipotassium oxalate monohydrate in 65 mL of water. C18 solid phase extraction cartridges (500 mg) were purchased from Biotage (Uppsala, Sweden). Bio-Beads S-X3 were purchased from Bio-Rad (Marnes-la-Coquette, France).

### 2.2. Standards

Chlordecone and $^{13}C_{10}$-chlordecone were obtained from Cambridge Isotope Laboratories (Tewksbury, MA, USA or equivalent), at 100 ng/μL in nonane. Other OCPs, aldrin, a-chlordane, g-chlordane, oxychlordane, trans-nonachlor, cis-nonachlor, p,p′-DDT, o,p′-DDT, p,p′-DDE, o,p′-DDE, p,p′-DDD, o,p′-DDD, dieldrin, endrin, HCB, pentachlorobenzene, a-HCH, b-HCH, g-HCH (=lindane), d-HCH, heptachlor, heptachlor epoxyde cis (=exo = B), heptachlor epoxyde trans (=endo = A), a-endosulfan, b-endosulfan, endosulfan sulfate, endrin aldehyde, endrin cétone, méthoxychlor and mirex (=perchlordecone) were also from the same provider. All reference standards were characterized by GC–MS before use in order to check their quality. Solutions of chlordecone were prepared in acetonitrile (ACN), after intermediary solutions in ethanol (EtOH), because of immiscibility between nonane and ACN. Native chlordecone solutions were prepared from 100 to 0.2 pg $\mu L^{-1}$ by successive dilutions. Labeled chlordecone solutions were prepared at 50 and 10 pg/μL for isotopic dilution. Calibration standard solutions were prepared at 0.2, 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500 pg/μL for native chlordecone and 10 pg/μL for labeled chlordecone. All the solutions were stored at +4 °C. Their stability was checked by comparing new and older solutions (bias < ±15%).

### 2.3. Gel permeation chromatography

An Alliance e2695 Liquid Chromatography System and a Fraction Collector WFC3 both from Waters (Milford, MA, USA) equipped with a Bio-Beads S-X3 column (37 cm × 24.4 mm i.d.) were used for gel permeation chromatography (GPC) separation. The mobile phase was an isocratic system of ethyl acetate/cyclohexane (1:1, v:v) at 5 mL $min^{-1}$.

### 2.4. GC–MS, GC–MS/MS and GC–HRMS

Gas chromatography coupled to tandem mass spectrometry (GC–MS/MS) was achieved using a Hewlett–Packard 7890 gas chromatograph (Palo Alto, CA, USA), equipped with a DB5-MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) coupled to a Waters Xevo TQ-S mass spectrometer (Milford, MA, USA) equipped with an atmospheric pressure chemical ionization interface used in positive mode. Corona discharge was set at 1.5 kV, cone voltage at 0 V, source temperature was at 150 °C, auxiliary gas flow at 400 L $h^{-1}$ and no cone gas was applied. Diagnostic signals were acquired in SRM with 271.8 > 236.9 and 273.8 > 238.9 transitions for chlordecone at collision energies of 15 and 20 eV, respectively. Transfer line temperature was set at 350 °C and nitrogen gas flow at 400 mL $min^{-1}$. Injection was performed in the splitless mode (1 min) at 250 °C. Constant flow was used at 1 mL $min^{-1}$. Oven temperature was 120 °C (1 min), 40 °C $min^{-1}$ up to 220 °C (1 min), 5 °C $min^{-1}$ until reaching 250 °C (0 min) and 40 °C $min^{-1}$ up to 300 °C (2 min).

An Agilent 6890 gas chromatograph (Palo Alto, CA, USA) equipped with two different columns, i.e. a HT8PCB column (60 m × 0.25 mm i.d., 0.25 μm film thickness) by SGE (Milton Keynes, United Kingdom) or a Rtx-1614 (15 m × 0.25 mm i.d., 0.1 μm film thickness) by Restek (Lisses, France), was coupled to a JEOL JMS-800D double sector mass spectrometer (Tokyo, Japan). Electron ionization was set at 70 eV, source temperature was set at 280 °C. At mass resolution of 10,000 (10% valley), *m/z* 271.8102

Download English Version:

https://daneshyari.com/en/article/7611647

Download Persian Version:

https://daneshyari.com/article/7611647

Daneshyari.com