Analytica Chimica Acta 767 (2013) 112–117

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

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

# Determination of dioxin-like polychlorinated biphenyls in 1 mL whole blood using programmable temperature vaporization large volume injection coupled to gas chromatogram and high-resolution mass spectrometry

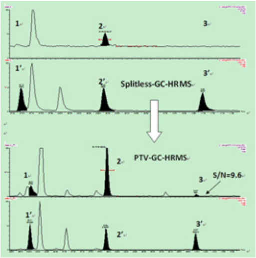
Haitao Shen[a,b], Rongfa Guan[c], Jingguang Li[a], Lei Zhang[a], Yiping Ren[b], Xiaomin Xu[b], Yang Song[d], Yunfeng Zhao[a], Jianlong Han[b,]**, Yongning Wu[a,]*

[a] Key Laboratory of Food Safety Risk Assessment, Ministry of Health; China National Center For Food Safety Risk Assessment, 7 Panjiayuan Nanli, 100021 Beijing, China
[b] Zhejiang Provincial Center for Disease Control and Prevention, 3399 Binsheng Road, 310051 Hangzhou, China
[c] Zhejiang Provincial Key Laboratory of Biometrology and Inspection & Quarantine, China Jiliang University, 310018 Hangzhou, China
[d] Zhejiang Academy of Medical Sciences, 182 Tianmushan Road, 310013 Hangzhou, China

## HIGHLIGHTS

- We developed and validated a PTV-GC–HRMS method to measure low level DL-PCBs.
- Liquid nitrogen was used in PTV external accessory equipment as fast cooling system.
- PTV's high-throughput and HRMS' high sensitivity and selectivity are well combined.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

*Article history:*
Received 13 October 2012
Received in revised form
24 December 2012
Accepted 5 January 2013
Available online 17 January 2013

*Keywords:*
PTV-GC–HRMS
Blood
Dioxin-like PCBs

## ABSTRACT

A sensitive method based on programmable temperature vaporization large volume injection coupled to gas chromatogram and high-resolution mass spectrometry (PTV-GC–HRMS) has been developed for the determination of ultra trace levels of dioxin-like polychlorinated biphenyls (DL PCBs) in small amounts of human blood. Blood samples (1 mL) were first extracted by column extraction and then purified with column chromatorgraphies. Final extracts (20 μL) were introduced to the PTV injector under the solvent vent mode and detected by GC–HRMS (SIM mode). PTV parameters were observed by changing one factor at a time (practical conditions: vent flow: 50 mL min$^{-1}$, vent pressure: 0 kPa and vent time: 0.1 min), recoveries of most PCB congeners ranged from 55.1% to 108%, and method detection limits were in the range of 0.11–1.63 pg g$^{-1}$.

## 1. Introduction

Polychlorinated biphenyls (PCBs) are internationally recognized persistent organic pollutants (POPs). These kinds of chemicals are environmentally stable and can accumulate in environmental matrices and biota due to their high lipophilicity. These pollutants are carcinogenic [1,2], and can adversely affect human health [3,4]. Biomonitoring can directly measure human body burden

---

* Corresponding author. Tel.: +86 10 83132933; fax: +86 10 83132933.
** Corresponding author. Tel.: +86 571 87115263; fax: +86 571 87115263.
*E-mail addresses:* jianlonghan@hotmail.com (J. Han), wuyongning@cfsa.net.cn (Y. Wu).

0003-2670/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2013.01.007

levels on internal exposure for more precise and realistic risk assessment. Among reported biomonitoring programs, adipose tissue and mother milk were the preferred medium for determining human exposure since they represent cumulative internal exposure. Blood was another suitable media that has been widely monitored because it is more easily obtained and represents more recent or non-work-related exposure [5].

The most reported instrumental techniques for POP analysis in blood include gas chromatography with electron capture detector (GC-ECD) [5–8], gas chromatography with negative chemical ionization mass spectrometry (GC-ENCI–MS) [9] and gas chromatography with high-resolution mass spectrometry (GC–HRMS) [10–14]. GC-ECD and GC–MS have good sensitivity and are affordable for most laboratories. However, they have a higher potential for detecting interfering components than do more selective analysis techniques. In contrast, although much more expensive, high-resolution mass spectrometry (HRMS) offers high sensitivity and selectivity, which results in less interference and improved accuracy. HRMS has long been considered as the confirmatory method.

POPs are lipophilic compounds and easily accumulate in lipid-rich tissues. Because the lipid content in blood is usually rather low (~0.5%, [14]), it is very difficult to use HRMS to detect these toxic compounds, such as 2,3,7,8-TCDD, at ultra low levels (*ppt* or lower) and in small sample sizes (<1 mL whole blood) simultaneously. To obtain reliable results, one alternative is to increase the sample size, for example 10–50 mL whole blood [11,15]. However, human samples are not always easy to obtain. For some special populations, such as children, collecting10 mL of blood is a big challenge for field sampling because children's veins are thinner than those of adults, and children's parents or schools often object to such a large collection volume. In the past, researchers have mixed samples with low lipid content together as pooled samples to obtain a sufficient amount of sample for analysis [13,16,17]. Another alternative is to further increase the method/instrumental sensitivity by utilizing a novel technique, such as programmable temperature vaporization large volume injection (PTV-LVI). Detailed principles of PTV (solvent vent mode) have been previously described [18,19]. In brief, PTV increases the injection volume of the final extracts and then gets rid of redundant solvent without loss of the target analyte to increase the overall sensitivity of the method. Application of PTV coupled to GC–MS or GC/MS/MS in POP analysis as screening and complementary methods to HRMS have also been well documented [19–22]. However, the application of PTV coupled to HRMS for POP analysis has not been reported yet. Thus, in the present work, we modified PTV external accessory equipment (added a liquid nitrogen cooling system) and further selected its parameters to make it work soundly with HRMS.

The aim of the present study was to develop and validate an analytical method that utilizes PTV-GC–HRMS for the determination of dioxin-like PCBs in a small amount of blood at ultra low levels. We expected the combination of PTV's high-throughput and HRMS's high sensitivity and selectivity would make PTV-GC–HRMS a more powerful tool for POP biomonitoring investigations.

## 2. Experimental

### 2.1. Chemicals and reagents

Calibration standard solutions: 68A-CVS, CS0.2-CS5, $^{13}$C-labeled surrogate standards: WP-LCS, injection standards: EC9605-RSand certified reference materials (CRMs; WMF-01) were obtained from Wellington Laboratories (Guelph, Canada). Column performance check solution (ED-935) was purchased from Cambridge Isotope Laboratories (Andover, USA). Hexane, dichloromethane (DCM) and other organic solvents were distilled-in-glass grade and purchased from Caledon Laboratories Ltd. (Ontario, Canada). Sodium sulfates were purchased from Sigma–Aldrich (Standard grade; Milwaukee, USA).

### 2.2. Sample collection

This study was approved by the Ethics Committee of the Zhejiang Provincial Center for Disease Control and Prevention. Blood samples were collected from a total of 15 Zhejiang residents at a community hospital in Hangzhou from May to June 2010. Before taking the blood, all volunteers have completed an informed consent form and an exposure assessment questionnaire. Samples were coded and frozen at $-20\,^{\circ}$C until analysis.

### 2.3. Sample extraction and purification

Whole blood (1 g) was spiked with $^{13}$C-labeled surrogate standards (WP-LCS) and then mixed with 50 g anhydrous sodium sulfate drop by drop in a mortar and immediately ground until the mixture was smooth. This mixture was then transferred into a glass column, and 100 mL hexane/dichloromethane (DCM) (1:1, v/v) was added to the column for at least 2 h of static soaking. The column was subsequently eluted with another 250 mL hexane/DCM. The extract was concentrated and purified by an acid/basic silica column and alumina column as described in our previous work [13].

### 2.4. Instrumental analysis

#### 2.4.1. GC/HRMS conditions

Dioxin like PCBs were analyzed by high-resolution GC/MS using an Agilent 6890 gas chromatograph coupled to an Autospec Ultima mass spectrometer (Waters Micromass, Manchester, UK) operating in EI mode at 35 eV and a trap current of 600 μA. The GC was equipped with a CTC PAL auto-sampler. An aliquot (20 μL) of the sample was injected into the PTV injector (Gerstel, Mühlheim, Germany) on a DB-5 MS fused silica capillary column (60 m × 250 μm I.D., film thickness 0.25 μm, J&W Scientific, Folsom, USA) with helium as the carrier gas at a constant flow rate of 1.4 mL min$^{-1}$. The oven temperature program was as follows: started at 40 °C, held for 1 min, increased to 115 °C at 65 °C min$^{-1}$, increased to 150 °C at 15 °C min$^{-1}$, increased to 270 °C at 3 °C min$^{-1}$, and held for 3 min, and finally increased to 310 °C at 15 °C min$^{-1}$ and held for 1.5 min. Quantification was performed using an isotope dilution method.

#### 2.4.2. PTV injector (solvent vent mode)

The PTV injector was operated insolvent vent mode. Liquid nitrogen (165 L capacity) was used to cool down the PTV injector after each sample cycle. The deactivated multi-baffled liner (1.5 mm I.D., 71 mm length and 150 μL capacity, Agilent Technologies) was modified by packing deactivated glass wool to help retain the high boiling point analyte. The syringe mode was large volume multiple injection. Specific parameters are discussed below.

## 3. Results and discussion

### 3.1. Selection of solvent vent PTV parameters

The PTV performance was influenced by several factors, including the solvent's boiling point, initial injector temperature, injection speed, solvent venting time, venting pressure and flow [18]. In this study, we used a univariable approach to observe the practical parameters. The principle was as follows: first, inject 1 μL analyte (ED-935) under the splitless mode and regard its peak area as a benchmark value (recovery = 100%); second, switch to