Determination of PCDD/Fs in Human Blood
A Fast and Sensitive Method

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Abstract

The development of a fast and sensitive method on the determination of polychlorodibenzodioxins (PCDDs) and polychlorodibenzufurans (PCDFs) in small amounts of human blood has been undertaken. The method has been applied with amounts of 5 ml whole blood and will be adapted for similar amounts of serum. The detection limit for 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD) is in the range of 1 pg/g blood lipids (1 ppt) and 10 - 20 fg/g original blood/serum respectively (10 - 20 ppq).

Introduction

In 1987 Patterson et al. (1) published a method for the determination of 2,3,7,8-TCDD in serum. The amount of serum analysed was 200 ml corresponding to 400 ml whole blood. Kahn et al. (2) and Nygren et al. (3) used 1988 the same amount of serum when presenting a method for the determination of all 2,3,7,8-substituted PCDD/F isomers. In 1989, Päpke et al. (4) reported the determination of all PCDD/F isomeres in blood using amounts of 40 g whole blood. The current "normal" HRGC/HRMS from Patterson et al., 1992, involves 50 - 200 ml serum (5, 6). The method is like all "normal blood methods" relatively time consuming and expensive.

Further reduction of the blood amounts to be analysed for PCDD/PCDFs have been reported by Patterson et al. recently (7). Patterson introduced the C2DGC (Comprehensive Two-dimensional Gas Chromatography System) and the fast gas chromatography in the determination of PCDD/F in blood.

Due to a project to analyse serum samples from infants (and therefore the limited amount of available blood) we started the development of a method to determine PCDD/PCDF in 4 - 5 g serum samples with adequate detection limits. The aim was not only to perform fast analyses but also - even more important - an increasing sensitivity.

Materials and Methods

The method described involves a separation of the blood lipids from a ChemElute/blood mixture by a solid-liquid extraction with a mixture of hexane/isopropanole. In this method some main steps have been changed compared to our established method (4).

The important steps of the analytical procedures are:

Preparation of a mixture of 5 g whole blood or serum, 3,5 ml water and 1 ml ethanol, spiked with all 17 2,3,7,8-substituted ^{13}C PCDD/PCDF standards.

After adding 2 g sodium chloride (NaCl) and 10 g of ChemElute followed by intensive mixing by shaking, the lipids are extracted by 4 portions (total of 100 ml) of a mixture of hexane/isopropanol (3 : 2).

After removal of water with sodium sulfate the solvents are carefully removed for the gravimetric lipid determination.

The redissolved lipids are transferred into a precleaned disposable carbon column (Amoco AX21/ Celite). After the elution of mono- and diortho PCBs with dichloromethane, planar PCBs and PCDD/PCDFs are eluted with toluene in reverse mode from the carbon column.

The final careful evaporation of the solvent is followed by adding of 5 µl toluene (containing ^{13}C-1,2,3,4-TCDD) and measurement by Fast GC/HRMS.

Experimental conditions:

Gas Chromatography		**Mass Spectrometry**	
Hp Hewlett Packard 5890 II		VG AutoSpec	
Column:	Fused silica, 15 m	R = 10.000	
	DB 5, 0,25 mm ID	Ion energy:	~ 30 eV
	0,1 µg Film thickness	Acc. Vol.:	8000 V
		Cycle time	
Pressure:	150 kPa	per group:	10 masses: 205
Injection amount:	2 µl splitless		
GC-program:	90 °C - 2 min isotherm		
	45 °C/min to 210 °C		
	10 °C/min to 275 °C		
	275 °C - 3 min isotherm		

Total GC/MS run time: 10 min

Results

The method is in the final development. Results and validation will be reported at the conference.

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