On-line Coupled LC-GC-MS for the Determination of Toxic Non-ortho PCB Congeners in Human Plasma

Eva Grimvall[1,2], Conny Östman[1] and Ulrika Nilsson[1]

1 National Institute of Occupational Health, Division of Analytical Chemistry, 171 84 Solna, Sweden

2 Stockholm University, Department of Analytical Chemistry, 106 91 Stockholm, Sweden

Introduction

Determination of the toxic non-ortho PCB congeners, CB-77 (3,4,3',4'-tetrachlorobiphenyl), CB-126 (3,4,5,3',4'-pentachlorobiphenyl) and CB-169 (3,4,5,3',4',5'-hexachlorobiphenyl) in complex samples requires extraordinary efforts and advanced analytical techniques due to the low levels of occurance as well as a large number of interfering compounds. A critical part in all applied methods for determination of these substances is the separation of the target compounds from the bulk of PCB. This is mostly performed by fractionation on carbon or pyrene liquid chromatography columns followed by off line gas chromatography - mass spectrometry. With off-line techniques, usually only a small fraction of the sample is injected into the GC. This is a major disadvantage when analysis near the limit of detection is to be performed.

When on-line coupled LC-GC utilizing concurrent solvent evaporation technique is applied, a larger fraction of the sample or the entire sample can be injected into the GC. Further benefits of the automated LC-GC system are that a part of the clean-up procedure and the final analysis is performed on-line, a more reproducible sample handling is obtained and it is possible to automate the clean-up/analysis by connecting an auto-sampler to the system. Previous use of on-line coupled LC-GC have been restricted to the determination of the most abundant congeners, utilizing e.g. a cyanopropyl silica HPLC column to purify the PCB fraction[1,2]. A modification of the LC-GC-MS system presented here has previously been used for the determination of chlorinated polycyclic aromatic hydrocarbons in ambient air[3].

At present, there are very few reports of non-ortho PCB congeners in human plasma Some determinations of non-ortho PCB in humans are performed on adispose tissue. However, that is very complicated when biological monitoring of groups of people is to be performed.

LC-GC-MS Instrument Set-up

HPLC-system

A dinitroanilino-propyl silica (DNAP) column (10 cm x 3 mm, 5 µm particles, ES Industries, Berlin, NJ) is used for separation of the toxic non-ortho PCB congeners from the bulk of PCB. On this column, these three toxic compounds elute as one single peak after the other congeners[4]. Pentane is used as mobile phase at a flow rate of 0.5 ml/min and a UV detector set at 254 nm is applied to monitor the eluent. The bulk PCB is collected from the the waste outlet and further analysed by off-line gas chromatography. When the non-ortho CBs start to elute, the flow is directed to a sample loop of 500 µl by automatic switching valves. After one minute, when the sample loop is filled, the eluent is redirected to the waste outlet and at the same time the flow direction through the HPLC-column is reversed in order to clean the column from remaining sample material.

LC-GC-interface

The HPLC heart-cut fraction of 0.5 ml is transferred to the GC by directing the carrier gas through the sample loop interface and further to a retention gap, figure 1. The retention gap is connected to a precolumn and then, through a T-piece to a solvent vapor exit and the analytical column respectively. During injection, the vapor exit is open and there will only be a very small flow through the analytical column. The carrier gas, helium, is controlled by a constant pressure regulator and a constant flow regulator in series. During injection, the constant flow regulator will raise to the highest pressure possible, set by the constant pressure regulator. The temperature of the column oven is held at 69 °C at a column head pressure of 0.78 bar. This causes the pentane to evaporate when entering the retention gap, i.e. concurrent solvent evaporation, while the PCBs will be retained in the retention gap or in the precolumn. The injection temperature has to be carefully adjusted according to the applied pressure. When injection is finished, the vapor exit is closed and a GC oven temperature program is started.

GC-MS

The GC separation column is a Rtx-5, 60 m x 0.32 mm, 0.25 µm film thickness. An injection temperature of 69 °C is held for two minutes after the finished injection and then the temperature is raised by 10 °C/min to 150 °C. The temperature is further raised by 2,5 °C/min to 250 °C, further by 10 °C/min to 280 °C, which is held for 5 minutes.

The transfer line to the mass spectrometer, an INCOS 50 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA) is maintained at 300 °C and the column outlet is directed into the mass spectrometer ion source which has a temperature of 150 °C. The instrument was operated in EI mode with an electron energy of 70 eV and with multiple ion detection monitoring the two most intense ions of the PCB congeners.

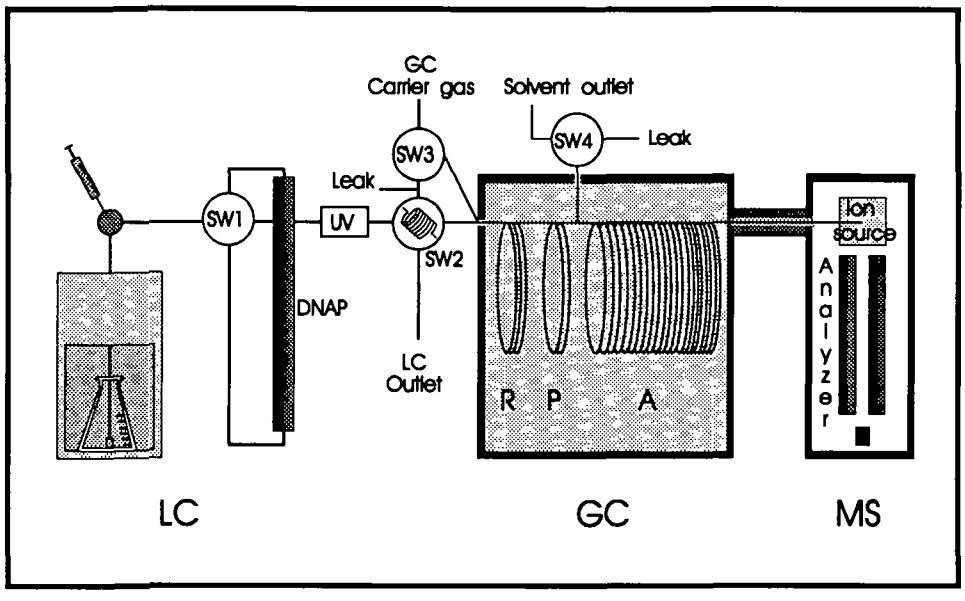

Figure 1: A scheme of the coupled LC-GC-MS system. Switch 1 (SW1) is used to control the flow direction of the HPLC mobile phase. Switch 2 (SW 2) puts the 500 µl loop interface in either the LC or the GC mobile phase stream. During sample transfer from the LC to the GC, switch 3 (SW3) directs the carrier gas through the loop interface. During GC analysis and GC stand by SW3 directs the carrier gas directly to the retention gap. Switch 4 (SW4) opens the solvent vapour exit during the concurrent solvent evaporation process. The two leak-capillarys are necessary to ventilate the system from remaining solvent vapours. R = retention gap. P = pre-column, A = analytical column.

Sample pre-treatment

The plasma samples are treated with an equal volume of formic acid and then extracted with hexane:diethylether (1:1). Lipid removal is performed by gel *permeation chromatography using Biobeads SX-3 and dichloromethane as mobile* phase. The solvent is evaporated and the sample dissolved in pentane prior to injection on the LC-GC-MS system. ^{13}C-CB 77 is used as an internal standard for the toxic non-ortho compounds and CB-58 is the internal standard for bulk PCB.

Results

Preliminary quantitative results of non-ortho CBs in 25 ml plasma samples from two students where for CB-126 0.5 and 0.9 pg/g plasma and for CB-169 0.5 and 1.0 pg/g plasma respectively while CB-77 was below the detection limit. The concentrations obtained for CB-126 and CB-169 are in the same orders as reported in litterature[5,6]. Work is in progress in order to further optimize and validate the presented method.

Acknowledgements

The project is financially supported by the Swedish National Environmental Protection Board and the Swedish Work Environment Fund.

References

1) *Grob K., Muller Jr. E. and Meier W. (1987): Coupled HPLC-GC for Determining PCB in Fish.* HRC&CC 10, 416 - 417
2) Hyvönen H., Auvinen T., Riekkola M-L. and Himberg K. (1992): Determination of Single PCB Congeners in Fish by Online LC-GC. J. Microcol. Sep. 4, 123 - 127
3) Östman C and Nilsson U. (1992): Coupled LC-GC-MS for On-line Clean-up, Separation, and Identification of Chlorinated Polycyclic Aromatic Hydrocarbons at Picogram Levels in Urban Air. HRC & CC. 15, 745 - 750
4) Grimvall E and Östman C. (1994): Fractionation of Non-ortho Substituted Toxic Polychlorinated Biphenyls on Two Nitro Containing Liquid Chromatography Stationary Phases. J. Chromatogr. Submitted
5) Pattersson D.G.Jr., Lapeza C.R.Jr., Barnhart E.R., Groce D.F. and Burse V.W. (1989): Gas Chromatographic / Mass Spectrometric Analysis of Human Serum for Non-ortho (Coplanar) and Ortho Substituted Polychlorinated Biphenyls Using Isotope-Dilution Mass Spectrometry. Chemosphere 19, 127 - 134
6) Asplund L., Svensson B-G., Nilsson A., Eriksson U., Jansson B., Jensen S., Widequist U. and Skerving S. (1994): PCB, p,p'-DDT and p,p'-DDE in Human Plasma Related to Fish Consumption. Submitted. In Svensson G.G. (1993) "Human Exposure to Certain Health Implications of Some Toxic and Essential Compounds in Fish". Thesis, Lund University, Sweden