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Evaluation of GC-ion trap-MS/MS methodology for monitoring PCDD/Fs in infant formulas

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Abstract

The application of high resolution gas chromatography in combination with low resolution mass spectrometry with electron ionization and MS/MS detection (HRGC-MS/MS) is tested for its use in the analysis of PCDD/Fs in infant formulas. Development of the analytical method was based upon EPA directrices and international recommendations. Calibration linearity was tested and average relative response for any native and labelled compound over the five-point calibration range below 14% was found. The precision and accuracy of the proposed analytical procedure are also presented. Results obtained are in agreement with EPA criteria. The method is applied to the analysis of a number of initial and follow-on milk based infant formulas. In general, HRGC-MS/MS constitutes an interesting method for the analysis of dioxins in such matrices.

Keywords: Polychlorinated dibenzo-*p*-dioxins; Dibenzofuran; Infant formulas; HRGC-MS/MS; Ion trap; Pressure pulses injection

1. Introduction

During the last decades special attention has been focused on two families of organochlorine compounds, the polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs). Dioxins, as they are commonly called, are compounds with similar chemical properties and are primarily formed as unwanted by-products in many industrial processes. These organic pollutants are of great concern due to their environmental persistence, toxicity and bioaccumulation through the food chain (Buckley-Golder et al., 1999).

Dietary intake is the main route (>90%) for human exposure to these toxicants (Safe, 1998; Schlummer et al., 1998). The highest exposure in human population occurs in infants via breast-feeding (Parzefall, 2002) due to the concentrations of these compounds in human milk and their higher food consumption in relation to the body weight (Päpke, 1998; Wittsiepe et al., 2001).

A considerable amount of data exits for concentrations of PCDD/Fs in human milk that reveals a decrease in levels over the last years. Although WHO recommends breast-feeding as the feeding choice for babies, infant formulas are an alternative to breast-milk that can play an important role in the infants diet, therefore their potential contamination with PCDD/Fs is of public concern and the need exits to a continued monitoring program.

However, information about substituted breast-milk at this concern is scarce (Päpke and Tritscher, 2000; FSA UK, 2004) maybe due to the fact of the complexity of the analysis of these contaminants in a complicated matrix such us powdered milk or infant formulas or because these products are produced just for a specific poblational group.

The complexity of the determination of these pollutants is mainly caused by the low levels at which these compounds occur and the large number of possible interferences and matrix effects disturbing the determination of these analytes (Liem, 1999a).

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There is a continuing need of reliable analytical methods to use in determining compliance with national regulations as well as international requirements. The reference analytical method for characterising PCDD/Fs in food samples (EPA, 1994) comprises a multi-step procedure: extraction with organic solvents, extensive clean up stages and the use of high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) as the determination procedure. However, HRMS is a very expensive technology, which requires highly-trained operators (Brochu et al., 1994).

Ion trap mass spectrometry (IT-MS/MS) has been reported as a valuable technique for improved selectivity in PCDD/F analysis (Liem, 1999b; Kemmochi and Tsutsumi, 2001). The high selectivity of MS/MS technique is due to its characteristic PCDD/PCDF fragment ions produced by the secondary ionization, so that the interference caused by the sample matrices can be minimized (Kemmochi et al., 2002). Results from different complex matrices analyzed with IT-MS/MS, such as fly ashes, soils and sediment materials (Fabrellas et al., 2004), fish, cream, eggs and cheese (Hayward et al., 2001), powdered milk, yolk and animal feed (Eppe et al., 2004) revealed a remarkable reliability when comparing with the results obtained from HRMS.

As for sensitivity, many efforts have been done to get lower detection limits either by optimising diverse parameters like the ionization conditions (Kemmochi et al., 2002), or by setting up to 26 parameters that control gas chromatography and mass acquisition conditions (Hayward, 2002). They have even injected higher amount of sample into the GC column (Eppe et al., 2004). Results revealed the MS/MS suitability as a screening method for dioxin monitoring at ppt levels and new improvements may also be done for the continuing development of this analytical methodology.

The aim of this study was to optimize HRGC-ion trap-MS/MS methodology with pressure pulses sample injection for the analysis of dioxins in a complex matrix such as powdered infant formulas. Subsequently, it was applied to the analysis of different trades of infant formulas.

2. Experimental

2.1. Chemicals

Solvents employed (*n*-hexane, acetone, *n*-pentane, dichloromethane, carbon tetrachloride and nonane) were of trace analysis grade (Lab Scan, Dublin, Ireland). Hexane was also glass distilled. Sulfuric acid (95–98%), sodium chloride and sodium hydrogen carbonate analytical reagent grade were purchased from Panreac (Barcelona, Spain). Aluminium oxide and silica gel were from Merck (Darmstadt, Germany). Celite® was from Fluka Chemika (Steinheim, Germany) and Isolute Sorbent® from Argonaut (United Kingdom).

Each batch of solvents was subjected to a solvent purity test for residue analysis suitability, according to the Association of Official Analytical Chemists recommendations (AOAC, 2005), and no interfering impurities were observed. Sodium sulfate (Na_2SO_4) anhydrous was purified by heating in a furnace at 500 °C for 6 h.

All PCDD/PCDFs standard solutions used: calibration and verification solutions (EPA 1613 CVS), spiking standard solution (EPA 1613 LCS) internal standard solution (EPA 1613 ISS) and the precision and recovery standard solution (EPA 1613 PAR) were purchased from Wellington Laboratories (Ontario, Canada).

2.2. GC-MS/MS instrumentation

All analysis were performed on a CP-3800 high resolution gas chromatograph (HRGC) equipped with a CP-8200 autosampler and coupled by a heated transfer line (280 °C) to a Saturn 2000 ion trap MS/MS spectrometer (Varian, Walnut Creck, CA, USA). Saturn GC/MS Workstation System software version 6.4 was used for data adquisition. Chromatographic separation was achieved with a Varian Factor Four fused-silica capillary column (50 m × 0.25 mm ID, 0.25 μm film thickness) with helium as the carrier gas at a linear velocity of 1 ml/min.

The retention time values for the 17 toxic PCDD/PCDFs on the column were established by injecting the CS3 calibration standard solution using full scan mode. The oven temperature program was 120 °C and held for 2 min, then to 200 °C (held for 3 min) at 30 °C/min, to 230 °C (held 15 min) at 3 °C/min and to 280 °C (held for 12 min) at 5 °C/min and finally to 310 °C/min (held for 3 min) at 10 °C/min. The chromatogram was obtained in 55 min.

Properly sample injection is basic for the chromatographic analysis and the sensitivity enhancement. During injection the vaporization of the solvent occurs, the split valve is opened and the sample is introduced into the chromatographic column set to a temperature below the boiling point of the solvent. Injection in the splitless injection mode (2 μl) with pressure pulses to a constant temperature of 300 °C was optimized and compared with injection without pressure pulses and ramped temperature.

Analysis by IT-MS/MS is a three step process where once the sample is injected and the chromatographic separation achieved, analytes undergo ionization. In our study, this first step was carried out by a beam of electrons. These beam of electrons, whose energy is varied to produce maximum ionization efficiency, collided with sample material inside the trap. This process is called ionization by electron impact (EI/MS/MS) or electron ionization. Then, a parent ion is selectively stored while all the other ions are removed from the trap. This step eliminates background while ensuring the selectivity. Finally, the isolated parent ions are fragmented into characteristic product ions through collision induced dissociation (CID), which yielded in a specific loss of the COCl fragment. The product ions were sequentially ejected from the trap according to their mass/