PCDD/F SCREENING AT THE MAXIMUM RESIDUE LEVEL FOR FOOD SAFETY ANALYSIS USING HIGHLY SELECTIVE TRIPLE QUADRUPOLE GC-MSMS

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Abstract
This paper presents a trace level screening method for PCDD/Fs and PCBs at the relevant maximum residue level for foodstuff. The method described employs a triple quadrupole mass spectrometer equipped with hyperbolic quadrupole rods for increased selectivity. The described MRM method is using selected MS/MS transitions for polychlorinated dioxins/furans and PCBs from two different precursor ions and detecting individual product ions for each chlorination degree. The analytical strategy follows the well-established United States Environmental Protection Agency (US EPA) Method 1613A by using isotope dilution quantitation with ^{13}C labeled internal standards.

Introduction
Polychlorinated dioxins (PCDDs/PCDFs) and polychlorinated biphenyls (PCBs) are amongst the biggest concerns related to food safety considerations. Considerable effort is taken worldwide to reduce global contamination with the effect of constantly decreasing levels found in feed and food. In contrast to lower general levels an increasing number of regional "accidents" in the food chain became public causing elevated levels of dioxins with worldwide impact (Irish pork, Italian mozzarella, and others).

In food production and control the continued low level screening is required to monitor the absence and compliance of raw materials as well as food products with current directives. In the European directives the maximum levels for dioxin and dioxin-like PCB contaminants are regulated for foodstuff, as well as the methods of analysis for screening and confirmation[1,2]. Requirements on performance of analytical methods applied are regulated by providing identification points to analytical techniques and their combinations[3]. Here the GCMSMS technique provides the potential for an instrumental screening method earning five identifications point when measuring two precursor ions with one product ion each, see Tab.1.

Technique	*Number of ions*	*Identification points*
GC-MS (EI or CI)	N	n
GC-MS (EI and CI)	2 (EI) + 2 (CI)	4
GC-MS (EI or CI) 2 derivatives	2 (Derivative A) + 2 (Derivative B)	4
GC-MS-MS	1 precursor and 2 product ions	4
GC-MS and HRMS	2 + 1	4
GC-MS-MS	2 precursor ions, each with 1 product ion	5
HRMS	N	2n

Tab 1.: Examples of the number of identification points earned for a range of GC-MS techniques (N,n = an integer)[3]

Sample screening requires the economical analysis of a large number of samples. While high resolution mass spectrometry is typically reserved for confirmation analysis of non compliant samples, alternative screening techniques as bioassays are discussed. Triple quadrupole GCMS uses similar sample preparation steps and offers the potential to overcome known false positive results of bioassays with a highly sensitive and reliable target compound quantitation at the required low trace levels.

Triple quadrupole GC-MSMS has turned out to become a standard trace analytical method for priority pollutants and pesticides in food and environmental analysis today. Using the highly selective precursor ion selection available with the Thermo Scientific TSQ Quantum GC using high precision hyperbolic quadrupole technology an increased selectivity for low level contaminants in matrix samples became available.

Materials and Methods

All sample analyses were carried out using the Thermo Scientific TSQ Quantum GC GC-MS/MS system, equipped with a Thermo Scientific TRACE GC Ultra™ gas chromatograph.

The TRACE GC Ultra was configured with a split/splitless injector. The sample introduction was performed using the Thermo Scientific TriPlus™ AS liquid autosampler. The capillary column was a Thermo Scientific TRACE™ TR-Dioxin 5MS column (5% phenyl film) of 30 m length, 0.25 mm inner diameter and 0.10 µm film thickness. Table 2 describes selected instrumental conditions for the employed Trace GC Ultra and TSQ Quantum GC mass spectrometer.

Tab.2: GC and MS instrument parameter

TRACE GC Ultra

Injector: Split/splitless, 260 °C, 1.2 min splitless
2 uL injection Carrier: He, constant flow, 0.8 mL/min Column type TRACE TR-Dioxin5MS, 5%-phenyl type Column: 30 m length, 0. 25 mm ID, 0.1 um film thickness, Temp. Program: 120 °C, 2 min
10 °C/min, 220 °C
220 °C, 2 min
3 °C/min, 260 °C
Transfer Line: 290 °C

TSQ Quantum GC

Source Temp: 250 °C, CEI volume Ionization: EI, 40 eV Emission Current: 100 µA Q1 Resolution: 0.7 Da Q3 Resolution: 0.7 Da Collision Gas: Argon, 2.0 mTorr Collision Energy: 22 eV

The optimization of the electron energy is critical for obtaining optimum results for dioxin detection. On the TSQ Quantum GC the optimum electron energy was determined with 40 eV for optimum sensitivity. This parameter should be determined once for a given instrument; typical optimum values are generally found between 40 and 50 eV.

The measurement protocol followed the US EPA Method 1613A with all required ^{13}C labeled internal standards (Wellington, Guelph, ON, Canada). The samples have been treated with internal standards at the time of sample clean-up for recovery and extract preparation (surrogate standard).

For data acquisition using the TSQ SRM mode the two most intense ions of the molecular chlorine isotope cluster of each congener and internal standard have been chosen, as described in earlier reports[4,5]. The multiple reaction monitoring sequence (MRM) was setup using retention time segments covering the congener elution of different chlorination degrees. Fig. 1 is describing the first retention time segment for tetra-chlorinated dioxins and furans.

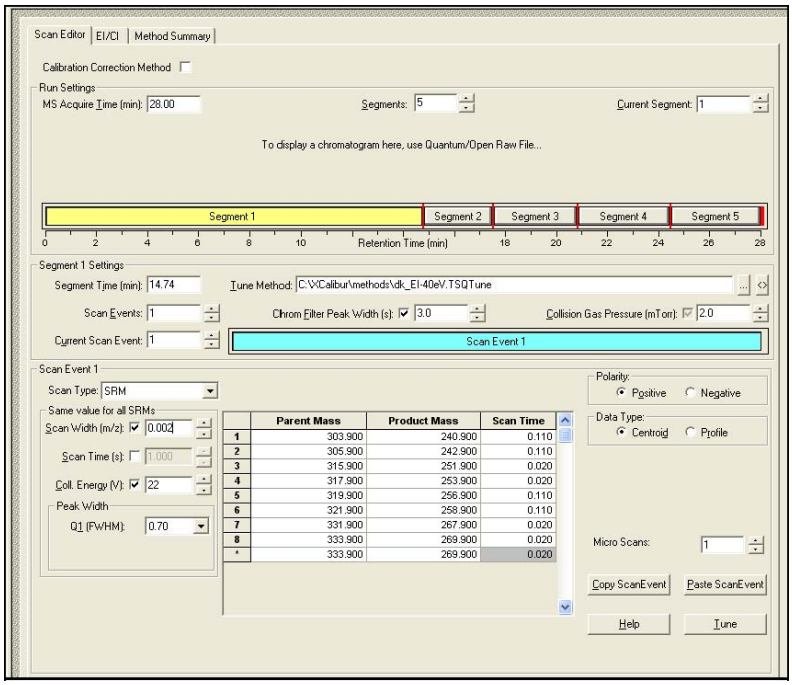

Fig. 1: TSQ Quantum GC acquisition parameters for the tetra-chlorinated dioxins and furans and internal labeled standards

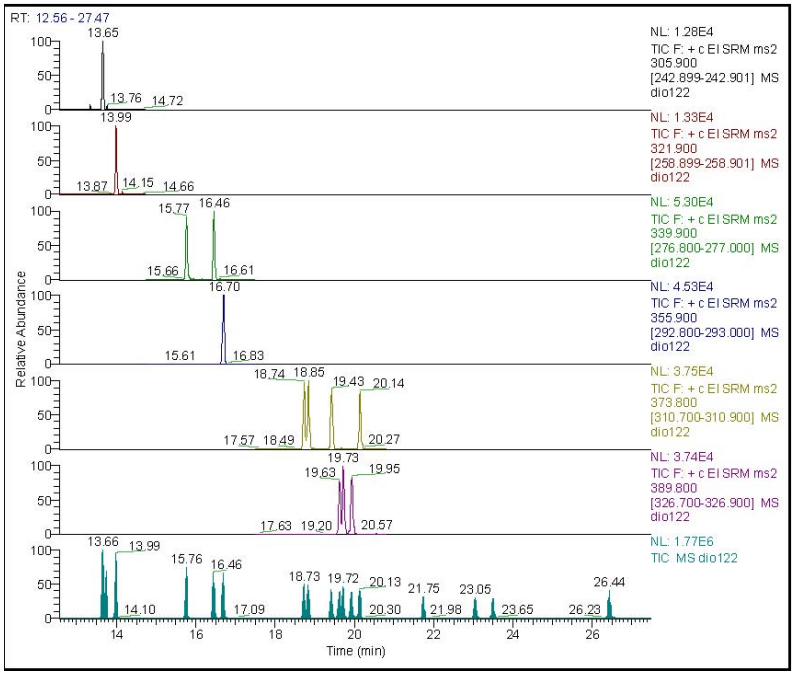

Fig. 2: EPA1613 CS1 standard 1/10 diluted; 2 ul injected, Quantum GC SRM mode (concentrations of natives: tcdd/tcdf: 50 fg/ul; penta to hepta dioxins/furans: 250 fg/ul; octas: 500 fg/ul)

Results and Discussion

The Thermo Scientific TSQ Quantum GC facilitates the screening and quantitation of polychlorinated dioxins, furans and PCBs at low levels in difficult matrix samples and provides results with high certainty. The added ^{13}C-labeled internal standard components can be detected with high reliability as has been demonstrated in different samples with complex matrix background (see a fish extract analysis in Fig. 2).

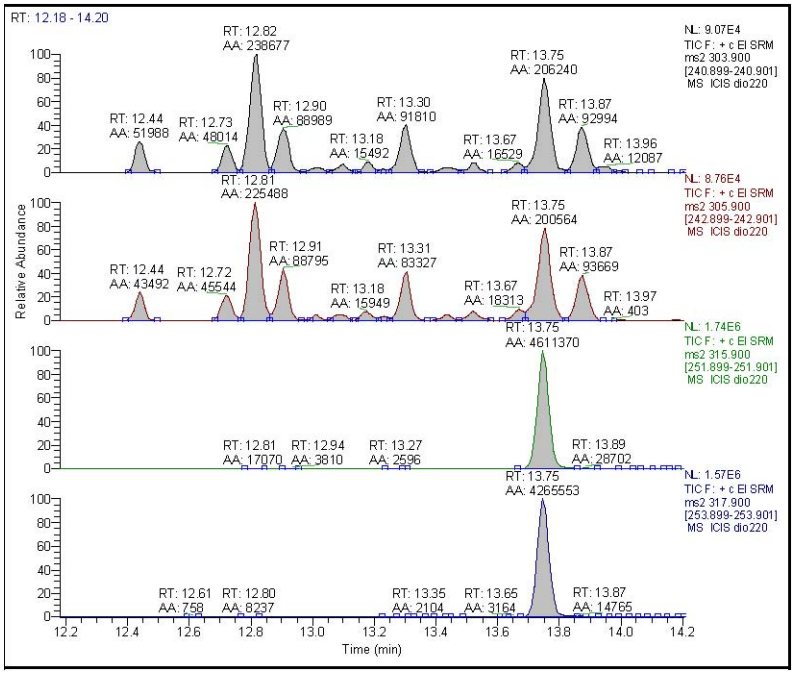

Fig. 3: Tetrafurans screenshot from a fish sample extract, 2ul injected. Top traces: major native peaks show mid fg range concentrations (500 – 600 fg tcdf). Bottom traces: labeled internal standard.

The TSQ Quantum GC with its unique hyperbolic quadrupole technology offers superior and uniform selectivity for low level dioxin and PCB samples in different complex matrices. See Figs 3 and 4 for the analysis of a fish sample. The proposed MS/MS measurement scheme using two precursor ions and SRM detection of individual product ions is a valuable solution for screening for dioxins/furans and PCBs in complex matrices at the relevant levels. For the fast control of food samples, GC-MS/MS with the TSQ Quantum GC exceeds the current EU directives for a minimum of four identification points, in that the method described here offers five identification points.

Safe screening methods using the TSQ Quantum GC cover the injected range at and above 100 fg absolute amount per compound injected which easily is accomplished by the currently used clean-up processes and injection techniques. Positive screening results, and to a lower extent negative results as well, have to be confirmed by a confirmatory method of analysis typically by high resolution mass spectrometry (HRMS) [2]. Modern HRMS systems as the Thermo Scientific DFS high resolution GCMS are capable of providing confirmatory results down to the very low fg level. With the high matrix selectivity and trace level sensitivity the TSQ Quantum GC provides a high productivity screening solution for increased sample throughput for food industry, contract and governmental control labs.

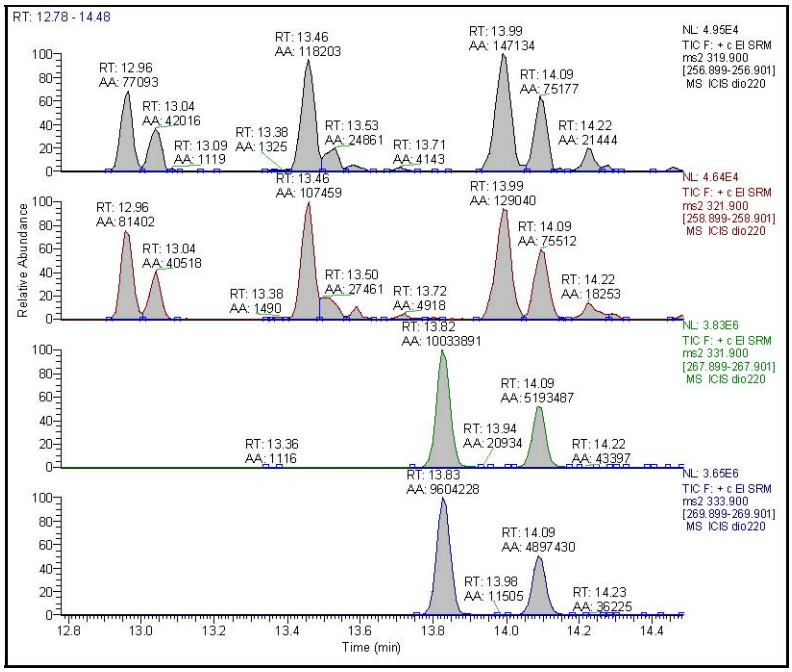

Fig. 4: Tetradioxin from a fish sample extract, 1ul injected. Top traces: major native peaks show mid fg range concentrations (200 – 300 fg tcdd). Bottom traces: labeled internal standards recovery and surrogate.

References

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