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PCDD/F Screening at the Maximum Residue Level for Food Safety Analysis using Highly Selective Triple Quadrupole GC/MS/MS

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Key Words

- EPA 1613A
- Food Safety Analysis
- Multiple Reaction Monitoring MRM
- PCB
- PCDD
- PCDF
- Screening

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 GC/MS/MS technique provides the potential for an instrumental screening method earning five identifications point when measuring two precursor ions with one product ion each, see Table 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

Table 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 GC/MS 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/MS/MS 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 XLS using high precision hyperbolic quadrupole technology an increased selectivity for low level contaminants in matrix samples became available.

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Materials and Methods

All sample analyses were carried out using the 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 XLS mass spectrometer.

TRACE GC Ultra	
Injector:	Split/splitless, 260 °C, 1.2 min splitless
Injection Volume:	2 µL injection
Carrier Gas:	He, constant flow, 0.8 mL/min
Column Type:	TRACE TR-Dioxin 5MS, 5%-phenyl type 30 m length, 0. 25 mm ID, 0.1 µm film thickness
Oven Temp. Program:	120 °C, 2 min 10 °C/min, 220 °C 220 °C, 2 min 3 °C/min, 260 °C
Transfer Line:	270 °C
TSQ Mass Spectrometer	
Source Temp.:	250 °C, CEI volume
Ionization:	EI, 40 eV
Emission Current:	100 µA
Resolution:	0.7 Da Q1, Q3
Collision Gas:	Argon, 2.0 mTorr
Collision Energy:	22 eV

Table 2: GC and MS instrument parameter

The optimization of the electron energy is critical for obtaining optimum results for dioxin detection. On the TSQ Quantum XLS 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. The acquisition of the different chlorination degrees is programmed most efficiently by using the sequential SRM mode. Only 5 segments are programmed to cover the relevant congeners and internal standards. The SRM transitions from precursor to product ions in each of the consecutive acquisition segments are given in Table 3.

Results and Discussion

The TSQ Quantum XLS 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 an EPA 1613 CS1 analysis in Figure 1).

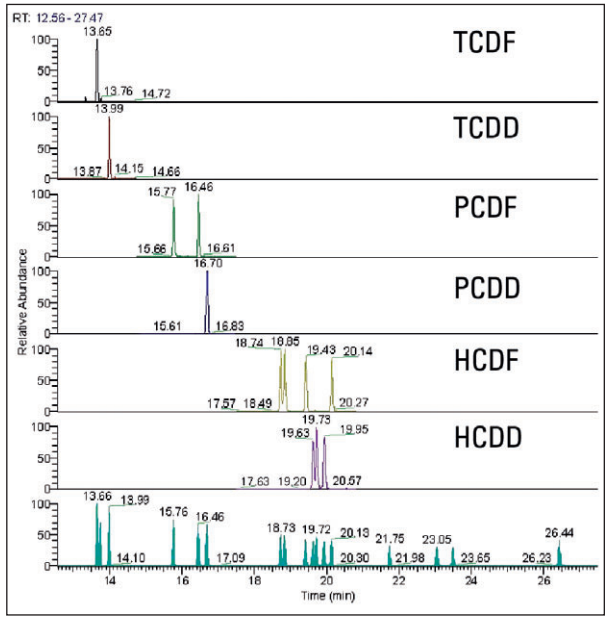

Figure 1: EPA1613 CS1 standard 1/10 diluted; 2 µL injected, Quantum GC SRM mode (concentrations of natives: tcdd/tcdf: 50 fg/µL; penta to hepta dioxins/furans: 250 fg/µL

The TSQ Quantum XLS with its unique hyperbolic quadrupole technology offers superior and uniform selectivity for low level dioxin and PCB samples in different complex matrices. See Figures 2 and 3 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, the method described here offers five identification points.

Safe screening methods using the TSQ Quantum XLS cover the injected range from below 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 XLS provides a high productivity screening solution for increased sample throughput for food industry, contract and governmental control labs.

Segment	Start (min)	Duration (min)	End (min)	Parent (*m/z*)	Product (*m/z*)	Dwell (s)	Compound
1	0	14.74	14.74	303.90	240.94	0.11	TCDF
				305.90	242.94	0.11	TCDF
				315.94	251.97	0.02	$[^{13}C_{12}]$TCDF ISTD
				317.94	253.97	0.02	$[^{13}C_{12}]$TCDF ISTD
				319.90	256.90	0.11	TCDD
				321.89	258.89	0.11	TCDD
				331.94	267.97	0.02	$[^{13}C_{12}]$TCDD ISTD
				333.93	269.97	0.02	$[^{13}C_{12}]$TCDD ISTD
2	14.74	2.74	17.48	339.86	276.90	0.13	PeCDF
				341.86	278.89	0.13	PeCDF
				351.90	287.93	0.02	$[^{13}C_{12}]$PeCDF ISTD
				353.90	289.93	0.02	$[^{13}C_{12}]$PeCDF ISTD
				355.85	292.85	0.13	PeCDD
				357.85	294.85	0.13	PeCDD
				367.90	303.90	0.02	$[^{13}C_{12}]$PeCDD ISTD
				369.89	305.89	0.02	$[^{13}C_{12}]$PeCDD ISTD
3	17.48	3.31	20.79	371.82	308.86	0.16	HeCDF
				373.82	310.86	0.16	HeCDF
				383.86	319.90	0.02	$[^{13}C_{12}]$HeCDF ISTD
				385.86	321.89	0.02	$[^{13}C_{12}]$HeCDF ISTD
				387.82	324.82	0.16	HeCDD
				389.82	326.82	0.16	HeCDD
				399.86	335.86	0.02	$[^{13}C_{12}]$HeCDD ISTD
				401.86	337.86	0.02	$[^{13}C_{12}]$HeCDD ISTD
4	20.79	3.64	24.42	407.78	344.82	0.17	HpCDF
				409.78	346.82	0.17	HpCDF
				419.82	355.86	0.02	$[^{13}C_{12}]$HpCDF ISTD
				421.82	357.85	0.02	$[^{13}C_{12}]$HpCDF ISTD
				423.78	360.78	0.17	HpCDD
				425.77	362.77	0.17	HpCDD
				435.82	371.82	0.02	$[^{13}C_{12}]$HpCDD ISTD
				437.81	373.81	0.02	$[^{13}C_{12}]$HpCDD ISTD
5	24.42	3.57	28.00	441.76	378.80	0.18	OCDF
				443.76	380.79	0.18	OCDF
				453.78	389.82	0.02	$[^{13}C_{12}]$OCDF ISTD
				455.78	391.81	0.02	$[^{13}C_{12}]$OCDF ISTD
				457.74	394.74	0.18	OCDD
				459.74	396.74	0.18	OCDD
				469.78	405.78	0.02	$[^{13}C_{12}]$OCDD ISTD
				471.78	407.78	0.02	$[^{13}C_{12}]$OCDD ISTD

Table 3: SRM tranistions for tetra to octa PCDFs/PCDDs

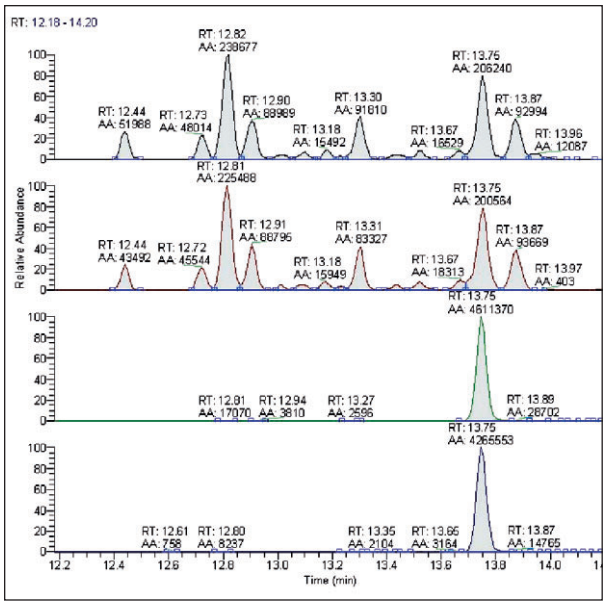

Figure 2: Tetrafurans screenshot from a fish sample extract, 2 µL injected. Top traces: major native peaks show mid fg range concentrations (500 – 600 fg tcdf). Bottom traces: labeled internal standard.

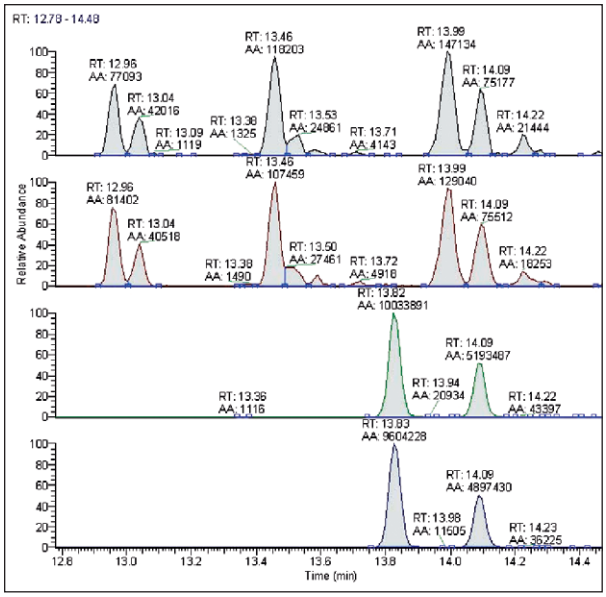

Figure 3: Tetradioxin from a fish sample extract, 1 µL injected. Top traces: major native peaks show mid fg range concentrations (200 – 300 fg tcdd). Bottom traces: labeled internal standards recovery and surrogate.

Conclusions

The Thermo Scientific TSQ Quantum XLS facilitates the screening and quantitation of polychlorinated dioxins, furans, and as well PCBs, at low levels in difficult matrix samples and provides results with high certainty. The proposed MS/MS measurement scheme using two precursor ions with detection of two congener specific product ions is a valuable solution for screening for dioxins/furans and dl-PCBs at the relevant MRL levels. For the fast control of food samples, GC-MS/MS with the TSQ Quantum XLS exceeds the EU directive with a required minimum of four identification points, in that the method described here offers five identification points.

Safe screening methods using the TSQ Quantum XLS cover the injected range at and above 100 fg absolute amount per compound which easily is accomplished by the currently used clean-up processes and available flexible injection techniques. Positive screening results, and to a lower extent negative results as well, have to be confirmed by a confirmatory method of analysis by high resolution mass spectrometry (HRMS). Such modern HRMS systems as the Thermo Scientific DFS High Resolution GC-MS provide confirmatory results down to the very low fg levels. With the high matrix selectivity and trace level sensitivity the TSQ Quantum XLS provides a high productivity and economical screening solution with high sample throughput for food industry, contract and governmental control laboratories.

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Original data acquired using the Thermo Scientific TSQ Quantum GC. Performance of the Thermo Scientific Quantum XLS typically meets or exceeds these results.

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