Triple-Quadrupole GC-MS/MS Technique for PCDD, PCDF and DL-PCB Determination in Milk

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Overview

Purpose: To develop a method to analyze PCDDs, PCDFs and DL-PCBs by a triple quadrupole GC-MS/MS system operating in the selected reaction monitoring (SRM) mode, to be used for a fast screening of dioxin-positive milk samples

Methods: Analysis was performed on buffalo milk and commercial cow milk. Samples were liquid/liquid extracted, subjected to lipid removal and cleaned-up on a multicolumn system. Quantitation was accomplished by the isotope dilution technique on a Thermo Scientific TSQ Quantum™ GC-MS/MS operated in EI SRM mode.

Summary:

• The TSQ Quantum GC system shows extremely good selectivity.

• Limits of quantitation (LOQs) in milk samples are in accordance with maximum levels set by EU Commission Regulations.

• Reproducibility (3 replicates) ranges up to 5.2%, recoveries range from 87 to 101%.

Introduction

As a consequence of an increased safety concern about dietary exposure to POPs, regulatory bodies such as the US Food and Drug Administration (FDA), the US Environmental Protection Agency (EPA) and the EU Commission authorities have produced detailed testing guidelines for dioxins and dioxin-like substances in food of animal origin. One of the most relevant dietary source of exposure is represented by milk and dairy products, for which provisional maximum levels (MLs) were established by the Regulatory Commission (EC) N° 199/2006 at 3.0 pg/g fat (PCDD+PCDF WHO-TEQ) and 6.0 pg/g fat (PCDD+PCDF+DL-PCB WHO-TEQ).

Determination of PCDDs, PCDFs, and DL-PCBs at regulatory levels requires very low limits of quantitation (LOQs), and hence requires highly selective and sensitive testing techniques. High resolution mass spectrometry (HRMS) based on magnetic sector instruments is the reference measurement method for confirmation of PCDD/Fs and DL-PCBs contents, because of its very high sensitivity and selectivity. However, reliable high sensitive screening methods for routine quality control are still lacking today.

In routine screening analysis in food and feed control alternative methods, mainly bioanalytical methods, are currently often used. These bioassay methods are reported to be very sensitive but suffer from the limitation of generating many false positive samples. Such found non-compliant levels of contamination from a sample screening are required to be analytically confirmed by using GC-HRMS according to the EC regulation 1883/2006.

A new approach for screening with the triple quadrupole GC-MS/MS technique is proposed to identify non-compliant samples thus reducing the number of false positive samples to be confirmed. The selected reaction monitoring (SRM) technique of triple quadrupole MS offers a structure related selectivity comparable to the accurate mass selected ion monitoring (SIM) technique in HRMS for matrix samples. Therefore, the triple quadrupole-based technique provides the potential for application in low level dioxin and DL-PCB screening.

Materials and Methods

Sample Preparation

50 mL of buffalo milk and commercial cow milk samples were spiked with 13C-labelled PCDD, PCDF, and DL-PCB internal standards and allowed to rest overnight at 4° C.

Before extraction, spiked samples were allowed to thaw at room temperature, added with calcium oxalate and methyl alcohol and then subjected to liquid–liquid extraction in centrifuge bottle with a mixture of diethyl ether and n-hexane by manual shaking, followed by centrifugation at 2000 rpm for 10 min.

The extraction was performed twice and the n-hexane aliquots collected from centrifugation were removed, pooled in an evaporation flask and carefully evaporated in a rotary evaporator until constant weight in order to gravimetrically determine the amount of the extracted lipids. The extract was then redissolved in n-hexane and subjected to lipid removal on an glass column containing Extrelut impregnated with concentrated sulphuric acid. After lipid removal cleanup was performed using the automatic Power-Prep system by way of three different pre-packed columns (multilayer silica, alumina, and graphitized carbon).

Instrumentation

A GC-MS/MS system TSQ Quantum was used for this study . The system comprised of a Trace GC Ultra™ gas chromatograph equipped with PTV injector, the TriPlus™ autosampler and interfaced with a TSQ Quantum triple quadrupole MS/MS detector operated in EI SRM mode.

TABLE 1. GC-MS/MS Instrumental Operating Conditions

Column	TR-5, 60 m length x 0.25 mm I.D. x 0.25 µm film thickness; Thermo Fisher Scientific, UK	**MS Transfer line**	290 C
Carrier gas	He, constant flow 1 mL/min	**MS Mode**	Selected Reaction Monitoring
PTV Injector	Mode: PTV Solvent Split Injection volume (µL): 5 Inject Time (min): 0.1 Base Temp (C): 110 Transfer Rate (C/s): 14.5 Transfer Temp (C): 300 Transfer Time (min): 1.2 Vent Flow (mL/min): 30 Splitless Time (min): 1.20 Split Flow (mL/min): 10	**GC Oven**	Initial Temp (C): 80 (1.10 min) Rate #1 (C/min): 40.0 Final Temp #1 (C): 230 (0 min) Rate #2 (C/min): 2.0 Final Temp #2 (C): 305 (0 min) Rate #3 (C/min): 30.0 Final Temp #3 (C): 340 (7 min)

Results

The GC TSQ Quantum system shows extremely good selectivity due to the SRM technique used during data acquisition. Such a selectivity allows to achieve very low limits of quantification (LOQs) not only in standard solutions but also in various matrices (see Table 2). In fact the detection limits in standard solutions and matrix samples do not differ significantly. Using this approach it is possible to detect PCDD+PCDF and PCDD+PCDF+DL-PCB (upper bound (UB) approach) in commercial cow milk sample with very low levels of contamination (0.58 and 1.2 pg WHO-TE/g fat respectively). The comparison between these results and the regulatory MLs shows that the sensitivity delivered by the instrument is adequate to meet the EC regulation requirements.

As documented in Table 3, the quantitative performance of the system is very good even at PCDDs + PCDFs levels close to the regulatory MLs.

Results, cont'd

The concentrations of analytes determined are expressed in the Table 3 as pg WHO-TE/g fat (corresponding to analytical concentrations 11.6–14.0 pg/g of milk fat). The difference (Δ = (UB–LB) x [(UB+LB)/2] - 1) between the UB (upper bound) and the LB (lower bound) is very low, ranging from 0.0% for DL-PCB to a maximum of 3.6% for PCDDs + PCDFs. This parameter documents very good reliability of the determination using the GC-MS/MS approach. The reproducibility determined during the analyses of 3 replicates of each sample ranges up to 5.2% RSD and recoveries were evaluated to be in the range of 87 to 101%.

TABLE 2.
LOQs (injected quantities in fg) for PCDDs and PCDFs in commercial cow milk.

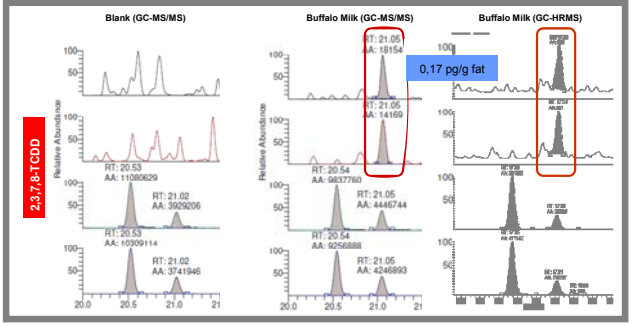
Analyte	LOQ	Analyte	LOQ
2,3,7,8-T_4CDD	16	2,3,7,8-T_4CDF	32
1,2,3,7,8-P_5CDD	32	1,2,3,7,8-P_5CDF	32
1,2,3,4,7,8-H_6CDD	80	2,3,4,7,8-P_5CDF	32
1,2,3,6,7,8-H_6CDD	80	1,2,3,4,7,8-H_6CDF	80
1,2,3,7,8,9-H_6CDD	80	1,2,3,6,7,8-H_6CDF	160
1,2,3,4,6,7,8-H_7CDD	80	1,2,3,7,8,9-H_6CDF	160
O_8CDD	200	2,3,4,6,7,8-H_6CDF	160
		1,2,3,4,6,7,8-H_7CDF	80
		1,2,3,4,7,8,9-H_7CDF	80
		O_8CDF	160

TABLE 3. Cumulative concentrations (pg WHO-TE/g fat) of PCDDs + PCDFs and DL-PCBs in buffalo milk samples (BM-A, BM-B, BM-C), n=3. UB: upper bound; MB: medium bound; LB: lower bound.

	BM-A	BM-B	BM-C	RSD%
PCDDs+PCDFs, UB	2.72	2.47	2.67	**5.2**
PCDDs+PCDFs, MB	2.76	2.50	2.72	**5.3**
PCDDs+PCDFs, LB	2.80	2.53	2.77	**5.4**
Δ%	**2.7**	**2.7**	**3.6**	
DL-PCBs, UB	1.10	1.11	1.11	**0.6**
DL-PCBs, MB	1.10	1.11	1.11	**0.6**
DL-PCBs, LB	1.10	1.11	1.11	**0.6**
Δ%	**0.0**	**0.0**	**0.0**	
PCDDs+PCDFs+DL-PCBs, UB	3.82	3.57	3.78	**3.6**
PCDDs+PCDFs+DL-PCBs, MB	3.86	3.61	3.83	**3.7**
PCDDs+PCDFs+DL-PCBs, LB	3.89	3.64	3.88	**3.8**
Δ%	**1.9**	**1.9**	**2.6**	

The extracted ion chromatograms shown in Figure 1 document the selectivity of detection achieved for TCDD at low pg/g levels in real samples (buffalo milk). The upper 2 traces in each picture show peaks of TCDD, lower 2 traces show peaks of isotopically labeled internal standards for each analyzed compound. It is clear that even at such a low concentrations (0.17 pg/g fat) the qualifier and quantifier peaks of TCDD can be easily identified and accurately integrated and quantified.

FIGURE 1. Chromatograms of 2,3,7,8-TCDD in blank and buffalo milk measured on GC-MS/MS (TSQ), and buffalo milk sample on HRMS (DFS, 1:10 dil.).



Comparison to GC-HRMS

During the work the comparison of results obtained by GC-MS/MS with those provided by HRMS on the same samples was carried out. In all cases very good agreement was observed (Table 4) confirming the applicability of the technique for high sensitivity dioxin screening purposes.

TABLE 4. Comparison of triple quadrupole GC-MS/MS and official method (HRMS) results (concentrations in pg WHO-TE/g fat), buffalo milk.

	GC-MS/MS	GC-HRMS	Δ%
PCDDs+PCDFs	2.70	2.63	**2.5**
DL-PCBs	1.11	1.20	**8.6**
PCDDs+PCDFs+DL-PCBs	3.81	3.84	**0.8**

Conclusions

GC-MS/MS was successfully applied to the analysis of low levels of PCDDs, PCDFs and DL-PCBs in commercial cow and buffalo milk samples. The results show that the performance of the technique is adequate to meet the EU regulations on screening methods. The data have been confirmed using the official confirmatory method GC-HRMS. By using the Thermo Scientific TSQ Quantum XLS GC-MS/MS system for screening, and DFS GC-HRMS for confirmation of positive results the complete EU regulations PCDD/F and POPs workflow becomes available.