

METHOD OF ANALYSIS VALIDATION OF DIOXIN-LIKE PCB'S IN DAIRY PRODUCT

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ABSTRACT

Dioxin like-polychlorinated biphenyls are unintentionally produced ubiquitous, persistent organic pollutants. The main source of human exposure to the compounds is food of animal origin because bioaccumulation in food chains. Extraction of dairy products by liquid-liquid extraction (LLE) technique for milk but extraction of other dairy products such as cheese was by soxhlet technique. In addition, clean samples after extracting the fat from dairy product samples by three - column chromatographyies, which include multi-layer silica, alumina column and then the third phase of the chromatographic column, is carbon. Method of analysis for determination of "dioxin-like PCBs" -show high toxicological properties that are similar to dioxins - in dairy products by high-resolution gas chromatography/ high-resolution mass spectrometry (HRGC/HRMS). Results were development of reliable and validated analytical method for dairy products, which recognized to be good indicator of environmental exposure for persistent organic pollutants. The method performance was tested for four non-ortho PCB congeners (PCB 77, 81,126, and 169) and eight mono-ortho PCB congeners (105, 114, 118, 123, 156, 157, 167, and 189) with good average recovery (via ¹³C12-labelled PCB internal standard) of the twelve PCB congeners for dairy products at level 40 ng/kg varies between 99-109 %. The trueness of a measurement method for dioxin like polychlorinated biphenyls in dairy product such as cheese where the results demonstrated satisfaction z-scores within range of ± 2 for sum non-ortho-PCB was -0.85, and for sum mono-ortho-PCB -0.74. The reproducibility expressed as relative standard deviation percent was less than 22.6 % and the measured uncertainty including random and systemic error (on 95% confidence level) was less than 40%.

Keywords: Method Validation, Dioxin Like PCB's, Liquid-Liquid extraction, Soxhlet, Dairy Product, Food and HRGC/HRMS

INTRODUCTION

The surveillance of dioxins and dioxin-like compounds in milk conducted since early 90's in many European countries. Raw milk and its products recognized to be a fine indicator of environmental exposure for persistent organic pollutants (POPs) (Lizak, 2003).

Lorber, M. *et al.* (1994) concludes the overwhelming dominance of the vapor phase transfers to vegetations which cattle consume, which in turn implies that the appearance of these chemicals in beef and milk is due to vapor transfers. Ruminants contaminated through vegetable feedstuffs, once dioxins absorbed by aerial deposition on the vegetation. Contaminants due to their lipophilicity and their low biodegradability bioaccumulation in cows, next they largely excreted from the body of lactating cows by transfer to milk. Milk and milk products are one of the major contributors to the human exposure to PCDD/Fs and PCBs. Moreover, during the last decade official monitoring

programmes revealed numerous dioxins crisis situations, particularly often in milk or beef meat (Malisch, 2000; Borrello et.al., 2008 and recommended by the European Regulation 1883/2006)

There is very limited information on the congener-specific concentration of U.K. cow's milk, with one study limited to ICES (International Council for the Exploration of the Seas) marker PCBs (PCB 28, 52, 101, 138, 153 and 180) in retail milk (Krokos et al., 1993).

There are distinct patterns in the results, which allow the congeners to classify according to their chlorine substitution pattern. The chlorine pattern of the PCB congeners analysed in the milk fat found at moderate and major concentrations in milk are typically substituted at both para positions (4, 4'), with the exception of PCB congeners, 155 and 126, which are found at trace levels in the environment generally. On the other hand, the PCB congeners not detected in the milk had at least one ring that not substituted. The following congeners routinely detected and quantified in milk fat: 28, 52, 61/74, 66, 101, 119, 110/77, 82/151, 149, 118, 188, 153, 105, 138, 187, 183, 128, 202/156, 180, 170, 194 (Sewart and Jones (1996)). This study validate the test method of analysis of DL-PCB's in dairy product using freeze-dried real samples, corn oil sample and inter-lab comparison (participated in 2011 on 12th round organized with 102 laboratories organized by Norwegian Institute of Public Health).

MATERIALS AND METHODS

1.0 Sampling:

A total of forty seven samples were included the corn oil sample and one mozzarella cheese as interlaboratory comparison and real contaminant of dairy product collected from domestic shops. Dairy products samples (Cheese, Butter, and Milk) extract of fat content from milk samples by liquid-liquid extraction (LLE) technique although the extraction of cheese made by soxhlet technique. Freeze-dried of homogenized fresh of milk and cheese samples were prepared before the two extraction techniques.

2.0 Chemicals and Reagents:

All solvents (toluene, cyclohexane, n-Hexane, methanol, methylene chloride, nonane and diethyl ether) used were from pesticide grade and purity not less than 99%. Silica gel was 0.063-0.200 mm, activated at 130 °C overnight, acid silica gel (30% w/w) and basic silica gel. Basic Alumina were from Aldrich (Brockmann I, standard grade, Milwaukee, USA). Anhydrous sodium sulphate (heated overnight at 300 °C and transferred to desiccator directly from 300 °C). Conc.H₂SO₄ (96%) from Riedel-deHaen. Carbopack 80/100 (Supelco), Potassium hydroxide pellets (MERCK) and celite-545 (BDH or Aldrich). Calibration standard solutions, labeled standard and injection solutions specified in EPA Method-1613B and EPA-1668 obtained from Cambridge Isotopes Laboratories (Andover, USA).

Reference Matrices Tissue reference matrix, corn or other vegetable oil in which the dl-PCB's detected by this method.

Precision and Recovery (PAR) Solution Wellington Laboratories Inc. (EPA 1668 PAR) for the dl-PCB's with certification to its concentrations used for determination of initial precision and recovery. Dilute 5 µL of the precision and recovery standard to 1.0 ml with acetone (dl-PCB's). One ml is required for the IPR with each batch.

Labeled-Compound Spiking Solution (LCS) Wellington Laboratories Inc. (EPA 1668) for the dl-PCB's with certification to its concentrations. Labeled-Compound spiking solution contains the dl-PCB's at the concentrations. Dilute 20 µL of the labeled compound standard solution to 1.0 ml with acetone (dl-PCB's). One ml is required for the IPR with each batch.

Internal Standard Solution (ISS) Wellington Laboratories Inc. (EPA 1668) for the dl-PCB's with certification to its concentrations. Internal standard contains 13C-PCB No. 52, 101, 138 and 194 in nonane at the concentrations for the dl-PCB's in nonane at the concentrations.

Calibration Standard Solution (CSS) Wellington Laboratories Inc. (EPA 1668 CS0.2-CS5) for the dl-PCB's with certification to its concentrations. These solutions permit the relative response (labeled to native) and response factor to measure as a function of concentration. The CS3 standard was used for calibration verification (VER).

3. Apparatus and Materials

3.1 Balance: 0.01g, with an accuracy of 0.001 g

3.2 Freeze-drying (lyophilization): Freeze Dryer iShin Lab co., Ltd.

3.3 Soxhlet Extractor (Bibby Sterlin, Great Britain): Soxhlet- 50 mm ID, 200 ml capacity with 500 ml flask Thimble- 43 x 123 to fit Soxhlet (Whatman or equivalent). Heating mantle Electromantle.

3.4 Cleanup Apparatus Anthropogenic isolation cleanup column: 300 mm long x 25 mm ID, with 300 ml reservoir. Silica gel and alumina cleanup columns: 200 mm long x 15 mm ID with 250 ml reservoir. Carbon cleanup column: 15 cm long x 6 mm ID.

3.5 Oven: baking and storage of adsorbents, in the range of 105-150 °C.

3.6 Concentration Apparatus Macro-Concentration (a rotary evaporator)-Heidolph or equivalent, Equipped with a variable temperature water bath. Sample and standard injection by use conical vials with 0.9 ml.

3.7 HRGC/HRMS Instrument Analyses were conducted using HP 6890 plus gas chromatograph coupled with Micromass /Autospec Ultima mass spectrometer operating in EI mode at 35 eV and with a resolution of 10.000 (5% valley). Sample injections performed in the splitless mode on DB5 MS column (60m, 0.25 mm id, 0.1µm film thickness). The oven program started from 90°C then takes 15min. to reach 220°C then held for 15 min, then from 220-290 in 8min then held for 17min. Helium (Ultra high purity) at a flow rate 0.8 ml/min. used as a carrier gas. Injector temperature was 225 C; 1µl of the sample injected using splitless mode.

4. Procedure:

4.1 Liquid-Liquid Extraction (LLE): Extract in triplicates freeze-dried of raw milk samples (100 ±0.1g) transferred to 1000 ml separator funnel; 4 ml of saturated solution of KOH added in order to digest the fat content; 200 ml of

ethanol and 100 ml of diethyl ether added (in order to denature the proteins). Fat extracted twice vigorously for 5 min with 140 ml of n-hexane. Inorganic phase discarded, while organic phase dehydrated with saturated solution of NaCl, twice. Finally, organic phase left with 50 g of Na₂SO₄ for final dehydration.

4.2 Soxhlet extraction: Extract in triplicates freeze-dried of cheese samples (25 ±0.1g) was extracted for 24 h in 200 ml n-hexane/dichloromethane (1/1, v/v) soxhlet extractors at the speed of six siphons per hour.

4.3 Five grams of dairy fat sample (e.g. butter) was heated up to 50 °C in an oven and afterwards filtered through filter paper to remove water and proteins included in butter, using clean-up without any extraction step.

The fat extracts were dried by filtration through 30-40 g of powdered anhydrous sodium sulfate, evaporated at 40°C using a rotary evaporator to lipid content determination using gravimetric analysis. Aliquots of about 1-7 g fat used for further step of clean up. Calculate the lipid content as following equation:

$$\text{Lipid Percent} = \frac{\text{Weight of residue (g)}}{\text{Weight of tissue (g)}} \times 100$$

Concentrate the extract to near dryness by using Macro-Concentration devise. Complete the removal of the solvent using the nitrogen blow down procedure and a water bath temperature of 60°C.

4.4 Cleanup The lipid was extract from the samples then transfer to cleanup steps. Cleanup column chromatography steps using acidified silica gel, anthropogenic, multilayer silica gel, alumina and active carbon column. After cleanup, the extract is concentrated to near dryness. Immediately prior to injection, injection standards added to each extract, and an aliquot of the extract injected into the gas chromatograph.

5. Determination of DL-PCB's Finally determine of DL-PCB's by using HRGC/HRMS. Quantitative analysis performed using selected Ion Recording (SIR) mode and the concentration of each compound is determined using the internal standard technique. The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and GC/MS systems. At the beginning of analyses, GC/MS system performance and calibration verified for all DL-PCB's and these labeled compounds. For these tests, analysis of the CS3 calibration verification (VER) standard perform until all performance criteria met such as blanks, analyze precision and recovery. Blank sample extracted with each tested sample in same batch immediately following tested samples aliquot to demonstrate freedom from contamination and freedom from carryover from the IPR analysis.

Determinations of DL-PCB's performed by an isotope dilution method using relative response factors previously obtained from five standard solutions. The TEQ concentrations were calculated guided to world health organization-toxic equivalent factor (WHO-TEFs, 1998), The DL-PCB's results for dairy product sample were identified and quantified and presented in pg WHO-TEQ/g fat weight (fw) multiplied by the associated WHO-TEF (Van den Berget *al.*, 1998). It assumed that non-detected isomer concentrations were equal to the limits of determination. As recommended by

the European Regulation (Council Regulation EC No. 199/2006), detection and quantification limits, as well as recoveries, for all DL-PCB's congeners were in good agreement with requirements laying down the sampling methods and the methods of the analysis for the official control of DL-PCB's. All steps of analysis conducted according to (U.S adverse consequences of the observed effects adverse consequences of the observed effects EPA 1613(B), 1994 and EPA 1668(B), 2008).

The QCAP lab operates and follows the quality assurance system and method of analysis of dl-PCB's in tissue and accredited since 2009 by Finnish Accreditation Service body (FINAS) according to the requirements of the International Standard ISO/IEC 17025. Moreover, the references of this method of analysis based on U.S. Environmental Protection Agency (EPA) Standard Method-1613 and 1668, and it is modifications to analyze dl-PCBs from biological samples.

RESULTS AND DISCUSSION

Quality Assurance/Quality control (QA/QC) by confirmation by method validation which includes of blank matrices, limit of detection and limit of quantization, precision and trueness such as Inter-laboratory comparison called proficiency test (P.T.). Eurachem guidelines (1998) followed in performing the different validation parameters and measurement uncertainty estimation.

Method validation for dioxins like PCB's analysis in dairy products
Blank matrices were analysed independently, in order to stand on the background levels of dl-PCB's, the result for the blank sample are represented in table (1).

Limit of detection (LOD) is the minimum concentration of analyte in the test sample that measured with a stated probability that the analyte is present at a concentration above that in the blank sample. The limit of detection estimated as three times of standard division of sample blanks fortified at lowest acceptable concentration measured once each. This approach assumes that a signal more than three times of standard division above the sample blank value could only have arisen from the blank. The limits of detection calculated for each congener as shown in table (1).

Limit of quantitation (LOQ) is the minimum concentration of analyte in the test sample that can be determined with acceptable precision (repeatability) and recovery under the stated conditions of the test. The lowest practical limit of quantitation estimated by using repeated spiked corn oil samples at the expected lowest quantitation level at 0.5 ng/kg. Accepted recovery and precision shown in the following table (1).

Table 1. LOD and LOQ estimated by repeatability of DL-PCB spiking solution (IPR) on corn oil sample

DL-PCBs		LOQ (Level of 0.5 ng/kg)				LOD (ng/kg)
		Mean	Rec.%	Sd	RSd(%)	
Non-Ortho PCB's Congeners	PCB 77	0.5	101	0.05	10.19	0.15
	PCB 81	0.49	97	0.03	5.83	0.09
	PCB 126	0.54	108	0.02	4.54	0.07
	PCB 169	0.54	108	0.06	11.16	0.18
Mono-Ortho PCB's Congeners	PCB 105	0.54	108	0.08	15.31	0.25
	PCB 114	0.54	109	0.05	8.71	0.14
	PCB 118	0.58	116	0.05	8.16	0.14
	PCB 123	0.52	104	0.07	12.65	0.2
	PCB 156	0.51	102	0.05	9.03	0.14
	PCB 157	0.56	113	0.02	2.84	0.05
	PCB 167	0.55	109	0.05	9.87	0.16
	PCB 189	0.5	101	0.01	2.59	0.04

No.: repeated samples = 10

Linearity for quantitative analysis, the range of analyte concentrations over which the method may apply determined by injection of six concentration levels (calibration solutions). The lowest calibration level was found to be 0.2 ng/ml and the highest calibration level was 2000 ng/ml. Six levels 0.2, 1, 5, 50, 400 and 2000 ng/ml are used for calibration.

Accuracy

Accuracy expresses the closeness of a result to a true value. Accuracy expressed in terms of two components: "Trueness" and "Precision"

Trueness is an expression of how close the mean of a set of results (produced by the method) to the true value. The method trueness tested by participated with 102 international laboratories, 12th round in 2011, which organized by Norwegian Institute of Public Health hence our lab (QCAP) have Lab No. 15. The satisfactory results conducted by Z-score for all dioxin like-PCBs congeners within the acceptable Z-score range (± 2) on natural contaminants levels in mozzarella cheese as shown in the table (2).

Precision is a measure of how close results are to one another. The two most common precision measures are (repeatability) and (reproducibility). The recovery tests for PCBs made by using repeated corn oil samples at different concentration levels, which called initial precision and recovery (IPR). The average recoveries and relative standard deviation on each level were calculated. The results of these experiments are shown in tables above (3 & 5).

Repeatability qualitatively is the closeness of agreement between successive results obtained with the same method on identical test material, under the same conditions (same operator, same apparatus, same laboratory and short intervals of time).

Table 2. Evaluation of dioxin Like PCB's analysis of Mozzarella Cheese, fresh weight from Inter-laboratory comparison on Dioxins in Food 2011 by using Soxhlet technique

Non- Ortho- PCBs	Reported Result Conc. pg/g fw.	Consensus median, pg/g	SD, pg/g	Z-Scores
PCB 77	1.2	0.6	0.33	1.82
PCB 81	0.39	0.42	0.12	-0.25
PCB 126	2.8	3.4	0.88	-0.68
PCB 169	1.1	1.2	0.26	-0.38
Sum Non-Ortho-PCB	0.291	0.35	0.0694	-0.85
Ortho- PCBs	Reported Result Conc. pg/g fw.	Consensus median, pg/g	SD, pg/g	Z-Scores
PCB 105	56	68	15	-0.80
PCB 114	7.1	8.3	2	-0.60
PCB 118	183	222	59	-0.66
PCB 123	3.5	3.8	0.93	-0.32
PCB 156	27	31	6.4	-0.63
PCB 157	7.2	8.2	2	-0.50
PCB 167	12	14	2.8	-0.71
PCB 189	4.7	5.3	1.4	-0.43
Sum Ortho-PCB	0.046	0.054	0.0108	-0.74

Z- Scores calculation = (Reported Result - Assigned value)/ St.dev

Repeatability experiments proceeding by fortification of dl-PCB on corn oil at different concentration levels as in recovery tests and its relative standard divisions were complying with EU requirement ($\leq 20\%$) shown in tables 3.

Table 3: The repeatability of DL-PCB's in corn oil samples at levels of 40 and 80 ng/kg.

DL-PCBs		Level of 40 ng/kg				Level of 80 ng/kg			
		Mean	Rec.%	Sd	RSd(%)	Mean	Rec.%	Sd	RSd(%)
Non-Ortho PCB's Congeners	PCB 77	42.263	106	1.87	4.42	83.47	104	6.04	7.24
	PCB 81	40.766	102	1.08	2.64	78.35	98	5.6	7.15
	PCB 126	43.77	109	1.86	4.24	82.87	104	3.2	3.87
	PCB 169	41.454	104	0.85	2.05	79.93	100	1.32	1.65
Mono- Ortho PCB's Congeners	PCB 105	41.505	104	1.23	2.97	83	104	8.17	9.84
	PCB 114	42.454	106	2.57	6.04	82.14	103	2.88	3.51
	PCB 118	39.597	99	3.31	8.36	86.26	108	9.64	11.18
	PCB 123	41.252	103	2.29	5.54	82.11	103	5.1	6.21
	PCB 156	40.264	101	1.52	3.78	79.03	99	3.15	3.99
	PCB 157	41.135	103	1.37	3.32	79.49	99	1.53	1.93
	PCB 167	40.994	102	1.18	2.88	80.49	101	0.93	1.15
	PCB 189	41.48	104	1.43	3.45	81.3	102	1.17	1.44

Sewart and Jones (1996) almost agreed the precision results of the method evaluated by analysis of five sub-samples of a bulked milk fat sample. The levels of precision were deemed satisfactory i.e. % relative standard deviation for PCB 118 and 180 were 0.6 and 2.2 respectively, and improved as the degree of volatility decreased and concentration of the congener increased. Recoveries of internal standards also found to be

acceptable, ranging between 45%-80%, losses attributed to the concentration/evaporation procedure.

Processed cheese sample used as a represented matrix for dairy products the sample purchased from local market. The repeatability experiments performed with; at least five replicates of real incurred dairy product sample by the same operator, same apparatus, same method and short intervals of time.

Table 4. The results of repeatability of dairy product samples as real contaminated samples

DL-PCBs		Repeated dairy product samples (ng/Kg)		
		Mean	SD	RSd(%)
Non-Ortho PCB's Congeners	PCB 77	0.39	0.07	18.53
	PCB 81	0.2	0.03	16.09
	PCB 126	0.2	0.03	14.26
	PCB 169	0.16	0.04	22.03
Mono-Ortho PCB's Congeners	PCB 105	2.93	0.34	11.66
	PCB 114	0.32	0.09	27.08
	PCB 118	9.07	3.51	37.73
	PCB 123	0.97	0.38	21.92
	PCB 156	1.15	0.22	18.64
	PCB 157	0.41	0.19	23.2
	PCB 167	0.66	0.06	19.78
PCB 189	0.19	0.04	20.38	
Sum Ortho+ non-ortho-PCB (WHO-TEQ)		0.024	0	12.64

No.: repeated samples = 6

Reproducibility considered spiking IPR test on corn oil samples analyzed by different analysts on several days. Reproducibility experiments for all congeners PCBs with ranging mean recovery and relative standard deviation from 107% and 16.3% for PCB 81 to 124% and 22.6 for PCB 118, respectively as shown in the table (5).

Table 5. The reproducibility of DL-PCB's in corn oil sample at level of 40 ng/kg

DL-PCBs		Repeated at level of 40 ng/kg (ng/Kg)			
		Mean	Rec.%	Sd	RSd(%)
Non-Ortho PCB's Congeners	PCB 77	47.17	118	5.66	12.0
	PCB 81	42.92	107	6.98	16.3
	PCB 126	44.62	112	6.88	15.4
	PCB 169	43.88	110	7.78	17.7
Mono-Ortho PCB's Congeners	PCB 105	48.33	121	5.55	11.5
	PCB 114	45.14	113	7.00	15.5
	PCB 118	49.68	124	11.24	22.6
	PCB 123	46.19	115	6.05	13.1
	PCB 156	45.16	113	5.15	11.4
	PCB 157	44.1	110	6.18	14.0
	PCB 167	44.45	111	6.80	15.3
PCB 189	43.54	109	9.20	21.1	

No.: repeated samples = 10

Measurement Uncertainty (U total)

The total uncertainty estimating the overall uncertainty, it may be necessary to take each source of uncertainty and treat it separately to obtain the contribution of each source. Each of the separate contributions to uncertainty referred to as an uncertainty component. The total uncertainty combined standard uncertainty, equal to the positive square root of the sum of the squares of the individual uncertainty components. The expanded uncertainty provides an interval within which the value of the measure and is believed to lie a higher level of confidence. Expanded uncertainty obtained by multiplying the combined uncertainty, by a coverage factor k, for confidence level of 95% k is 2.

Relative Standard Uncertainty The following equations used for relative standard uncertainty calculations in;

$$S = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n - 1}} \qquad Rsd\% = \frac{S}{x} \times 100$$

Where: S, is the standard deviation
 Rsd%, relative standard deviation
 \bar{x} , the average of n samples

The precision was estimated using results arise from the daily analyzed control samples; in this case the variation due to sample processing must be accounted for this gives a value for the relative standard uncertainty due to run variation of the overall analytical process.

Relative standard uncertainty due to precision (Uprecision) comes from spike samples were ranged between 11.4 - 22.6% for DL-PCBs.

Combined Uncertainty (Ucomp) Combined uncertainty, is the positive square root of the sum of the squares of different uncertainty components. Combined uncertainty found to be 20%. The following equation used for combined uncertainty calculations;

$$U_C = \sqrt{(U_{precision})^2 + U_{Ref}}$$

Expanded Uncertainty (U exp) Expanded uncertainty is obtained by multiplying the combined uncertainty by a coverage factor k, for confidence level of 95% k is 2 was found to be less than 40% as total uncertainty for dl-PCB's in dairy product shown in table (6).

Table (6). Summary of uncertainty results for DL-PCB's congeners in dairy product

#	DL-PCBs	Mean	Rec. %	RSD	U comp	U exp	U total*
1	PCB 77	47.17	118	0.1200	0.1032	0.2064	20.6
2	PCB 81	42.92	107	0.1627	0.1522	0.3044	30.4
3	PCB 126	44.62	112	0.1543	0.1387	0.2774	27.7
4	PCB 169	43.88	110	0.1773	0.1612	0.3225	32.2
5	PCB 105	48.33	121	0.1148	0.0952	0.1904	19.0
6	PCB 114	45.14	113	0.1550	0.1410	0.2821	28.2
7	PCB 118	49.68	124	0.2263	0.1930	0.3861	38.6
8	PCB 123	46.19	115	0.1310	0.1172	0.2344	23.4
9	PCB 156	45.16	113	0.1141	0.1018	0.2036	20.4
10	PCB 157	44.1	110	0.1401	0.1277	0.2555	25.5
11	PCB 167	44.45	111	0.1530	0.1380	0.2761	27.6
12	PCB 189	43.54	109	0.2113	0.1941	0.3883	38.8

No. of repeated samples= 10

*Uncertainty Measurement values at confidence 95%

Conclusions

DL-PCBs determined by isotope dilution using HRGC/HRMS in method blanks, initial precision and recovery (IPR), certified reference material and natural contamination from the domestic of shops. The entire methodology validated by analyzing 12 dl-PCBs in inter-laboratory comparisons with international laboratories organized by Norwegian Institute of Public Health with satisfaction results for all PCB's congeners. This Method validation is the process used to establish a quantitative analytical method is suitable for 12 dl-PCBs. Reassurances as to the quality of the method and its reliability come from adopting a minimum series of validation experiments and obtaining satisfactory results.

REFERENCES

- Borrello S, Brambilla G, Candela L, Diletti G, Gallo P, Iacovella N, Iovane G, Limone A, Migliorati G, Pinto O, Sarnelli P, Serpe L, Scortichini G, and di Domenico A, 2008. Management of the 2008 "buffalo milk crisis" in the Campania Region under the perspective of consumer protection. *Organohalogen Compounds*, 70, 891-893.
- EC (Commission Regulation), No. 199/2006 of 3 February amending Regulation No. 466/2001 setting maximum levels for certain contaminants in foodstuffs as regard to dioxins and dioxin-like PCBs. *Off J Eu Union*, L 2006; 32/34.
- EC (Commission Regulation), 1883/2006 of 19 December 2006. laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs. *Off. J. Europ. Union* L 364/32.
- EPA (Environmental Protection Agency, U.S.A) Method 1613, revision B "Tetra through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS". 1994.

- EPA (Environmental Protection Agency, U.S.A) Method 1668, revision B - Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS - November 2008.
- EURACHEM Guide (1998) The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics. LGC, Queens Rd., Teddington, Middlesex, TW110LY, United Kingdom. ISBN: 0- 948926-12- 0, Edition 1.0
- Krokos, F., Creaser, C.S., Startin, J.R. and Wright, C. (1993) The development and validation of a congener specific method for concurrent determination of ortho and non-ortho PCBs and dioxins. Poster presented at Dioxin, 93, Vienna, Austria.
- LIZAK, R., 2003. Development and optimization of extraction procedure of milk fat for simultaneous determination of dioxins and dioxin-like compound, Proceedings of ECOpole, Vol. 3, No. 2, 335-340
- Lorber, M., Cleverly, D., Schaum, J., Phillips, L., Schweer, G. and Leighton, T. (1994) Development and validation of an air-to-beef food chain model for dioxin-like compounds, vol.156, 39-65
- Malisch, R., 2000. Increase of PCDD/F-contamination of milk, butter and meat samples by use of contaminated citrus pulp. Chemosphere 40, 1041-1053.
- Sewart, A. and Jones, K.C. (1996) A survey of PCB congeners in U.K. cows' milk, Chemosphere, Vol. 32, No. 12, pp. 2481-2492.
- World Health Organization (WHO) (1998) WHO experts re-evaluate health risks from dioxins. Press Release WHO/45, 3.
- Van den Berg, M., L.S., Birnbaum, A.T.C., Bosveld, B., Brunstrom, P., Cook, M., Feeley, J.P., Giesy, de A., Hanberg, R., Hasegawa, S.W., Kennedy, T., Kubiak, J.C., Larsen, F.X.R., Van Leuwen, A.K.D., Liem, C., Nolt, R.E., Peterson, I., Poellinger, S.h., Safe, D., Schrenk, D., Rillitt, m., Tysklind, M., Younes, F., Waern and T., Zachewski (1998) Toxic equivalency factors (TEFs) for PCB's, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect, 106(12), 775-792.

طريقة التحقق من صحة تحليل مشابهات الديوكسين - ثنائي الفينيل متعدد الكلور في منتجات الألبان

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مشابهات الديوكسين- ثنائي الفينيل متعدد الكلور أحد الملوثات العضوية الثابتة والتي تطلق في كل مكان عن غير قصد. والمصدر الرئيسي لتعرض الإنسان لتلك المركبات تكون في الأغذية ذات المنشأ الحيواني وذلك بسبب تراكمها في السلسلة الغذائية. إن تقنية استخلاص منتجات الألبان تكون عن طريق تقنية الاستخلاص السائل- بالسائل (LLE) لعينات الحليب واستخدام تقنية الاستخلاص بالسوسكليت لمنتجات الألبان الأخرى مثل الجبن. يضاف إلى ذلك تنظيف العينات بعد استخلاص الدهون بأعمدة كروماتوغرافي، والتي تشمل كروماتوغرافي سائل متعدد الطبقات السيليك، ثم عامود الألومينا والمرحلة الثالثة للكروماتوغرافي هو العامود الكربوني. طريقة التحليل لتقدير مشابهات الديوكسين- ثنائي الفينيل متعدد الكلور، تظهر خصائص السمية العالية لتلك

المركبات في منتجات الألبان عن طريق جهاز كروماتوجرافي الغازي عالية الدقة / مطياف الكتلة عالية الدقة (HRGC/HRMS). وكانت نتائج تطوير طريقة التحليل والوثوق بها والتحقق من صحتها في منتجات الألبان، والتي ستكون مؤشرا جيدا عن مدى التعرض البيئي لتلك للملوثات العضوية الثابتة. تم اختبار طريقة أداء للأربع متجانسات لتلك المركبات غير-أورثو (PCB 77، 81126، و 169) والثمان متجانسات أحادية - أورثو (105، 114، 118، 123، 156، 157، 167، و 189)، حيث أعطت متوسطات استرجاع عالية (عبر إضافة مركبات قياسية بها ذرات كربون معلمة اشعاعا) يتراوح ما بين 99-109%. وذلك اجميع المتجانسات الأثنا عشر في منتجات الألبان عند مستوى تركيز 40 نانوغرام / كيلوغرام. تم التحقق من صدق طريقة تحليل مشابهاة الديوكسين - ثنائي الفينيل متعدد الكلور في منتجات الألبان مثل الجبن حيث أظهرت النتائج درجة رضاء ممثلة بقيمة Z ضمن مدى $2 \pm$ وقيمتها لمجموع غير-أورثو-ثنائي الفينيل متعدد الكلور هو 0.85-، وبالنسبة لمجموع أحادية - أورثو - ثنائي الفينيل متعدد الكلور هو 0.74-. دقة نتائج التحليل والتي تم تمثيلها بواسطة نسبة قيمة الانحراف المعياري ووجد انها أقل من 22.6%. وبقياس درجة عدم اليقين للطريقة والتي تشمل قياس الخطأ العشوائي والخطأ النظامي (عند مستوى ثقة 95%) ووجد انه أقل من 40%.

قام بتحكيم البحث

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