Analysis of Dechlorane Plus and related norbornene-based flame retardants in foods by gas chromatography - high resolution mass spectrometry

Jekaterina Rjabova[1,2], Arturs Viksna [2], Dzintars Zacs[1]

[1] Institute of Food Safety, Animal Health and Environment ''BIOR'', Lejupes iela 3, Riga, LV-1076, Latvia

[2] University of Latvia, Jelgavas iela 1, Riga, LV-1004, Latvia

Introduction

After EU imposed a ban on the marketing and use of technical polybrominated diphenyl ether (PBDE) formulations, norbornene-based flame retardants such as Dechlorane Plus (DP) and dechlorane–related compounds (DRCs) were regarded as essential alternatives [1]. A number of studies confirmed the presence of DRCs in various environmental objects [2,3] and food products [4,5]. However, human exposure to DRCs via food consumption is still poorly understood and thus the development of reliable methods for their analysis is one of the essential steps to implementing risk assessment strategies. This paper presents an analytical method for the determination of ten DRC representatives in various food samples by using an advanced non-destructive sample preparation procedure, which allows to include acid-labile compounds in the scope of the analysis. Analyte detection with GC-magnetic sector HRMS ensures high selectivity and sensitivity of measurements, while isotope dilution with ^{13}C-labeled surrogates and internal standardization provides reliable quantification of the compounds of interest. The developed method was extensively validated and applied for the analysis of numerous matrices representing various food types.

Materials and methods

Chemicals and materials

Target analytes and isotopically labeled internal standards were from Cambridge Isotope Laboratories (Tewksbury, MA, USA), Wellington Laboratories (Guelph, ON, Canada), Santa Cruz Biotechnology (Dallas, TX, USA) or from AccuStandard (New Haven, CT, USA). Organic solvents were of analytical grade and were purchased from Sigma-Aldrich (Buchs, Switzerland). Strata SI-1 Silica 500 mg / 6 mL SPE cartridges were obtained from Phenomenex (Torrance, CA, USA), while the Bio-Beads SX3 (200-400 mesh) sorbent was purchased from Bio-Rad (Philadelphia, PA, USA).

Sample preparation

Aliquots of freeze-dried samples were spiked with $^{13}C_{10}$-labeled internal standard solution. The mass of the aliquot depended on lipid content in the sample and was equivalent to approximately 1 g of lipid. The samples were extracted in a Soxtec™ 2055 Fat Extraction System (Hillerød, Denmark) with 1:1 DCM / *n*-hexane mixture. After the evaporation of extracts, high molecular compounds were removed using LC Tech Freestyle™ GPC system (Dorfen, Germany) on a glass column (50 × 2.5 cm) filled with 50 g of Bio-Beads SX3 stationary phase using 1:1 cyclohexane / ethyl acetate as eluent. The evaporated extracts were dissolved in 1 mL of cyclohexane and applied on the top of Strata Silica 500 mg / 6 mL cartridges, which were preconditioned with cyclohexane. The target analytes were eluted with 10 mL of cyclohexane, concentrated to dryness under a gentle stream of nitrogen, and reconstituted in 50 µL of recovery standard $^{13}C_{12}$-PCB 194 solution in nonane prior to instrumental analysis.

Instrumental analysis

The measurements were performed on a Micromass AutoSpec HRMS system (Milford, MA, USA) coupled to an Agilent 6890N gas chromatograph (Santa Clara, CA, USA). GC separation was carried out on a DB-5MS capillary column (30 m × 0.25 mm × 0.1 µm, Agilent Technologies). Acquisition was performed using selective ion monitoring (SIM) mode with resolution of >10,000 at 10% valley definition. Helium was used as the carrier gas at a constant flow rate of 1 mL min^{-1}. The GC oven temperature program started with isothermal period for 3 min at 100°C, than ramped to 315°C at 10°C min^{-1} and finally was held at 315°C for 15 min. The GC-MS transfer line and injection port temperatures were set at 280 and 310°C, respectively. The ion source temperature was set at 270°C and ionization was performed in positive electron impact mode (EI^+) with electron energy of 35 eV. The analytes of interest were detected using a three-segment MS acquisition method by monitoring the two most intense fragments from the molecular ion cluster (Table 1).

Table 1.

GC-HRMS characteristics for the determination of target analytes on DB-5MS column

Compound	RT, min	Time segment	Qual. ion**, m/z	Quan. ion*, m/z	Isotopic ratio
1,3-DPMA	15.48	1	265.8618	263.8648	1.56
Mirex	18.15	1	273.8072	271.8102	1.25
Dec 602	19.27	2	273.8072	271.8102	1.25
DBHCTD	19.51	2	273.8072	271.8102	1.25
Dec 603	21.79	3	264.8549	262.8570	1.56
Dec 604	22.17	3	417.7026	419.7006	1.46
Cl10DP	22.57	3	203.8881	201.8911	0.78
***Syn*-DP**	23.43	3	273.8072	271.8102	1.25
Cl11DP	23.68	3	239.8462	237.8491	1.56
***Anti*-DP**	23.91	3	273.8072	271.8102	1.25
$^{13}C_{10}$-Dec 602	19.25	2	276.8269	278.8240	1.25
$^{13}C_{10}$-*syn*-DP	23.42	3	276.8269	278.8240	1.25
$^{13}C_{10}$-*anti*-DP	23.90	3	276.8269	278.8240	1.25
$^{13}C_{12}$-PCB 194	18.97	2	439.8038	441.8008	1.12

Results and discussion

Method optimization

In order to reduce the consequences of suboptimal focusing and charging effects in the ion optics of the MS system, the acquisition method was divided into three time windows in such a way that each subsequent function contained fragments with nearly equal or higher *m/z* ratio than in previous time segment. Electron impact energy of 35 eV was found to provide the highest response under the conditions of EI^+ for all DRCs except for Dec 604, which had the response maximum at 45 eV. Nevertheless, electron impact energy of 35 eV was used in the optimized method, as it provided higher sensitivity for the majority of analytes. Three GC columns were tested for the separation of analytes (DB-5MS, DB-5HT, and Rtx-1614). All columns had the dimensions of 30 m × 0.25 mm × 0.1 µm. DB-5MS was found to be the most suitable, providing optimal sensitivity and repeatability of response. The elution profiles of analytes and high molecular compounds from the GPC column showed negligible overlapping, potentially causing some losses (<7%) of Dec 603 and *anti*-DP during the GPC clean-up. These fluctuations were compensated by using $^{13}C_{10}$-*anti*-DP as internal standard for both compounds. Three SPE cartridge types (Strata Florisil 500 mg / 6 mL, Strata SI-1 Silica 500 mg / 6 mL and Strata SI-1 Silica 1000 mg / 6 mL) in combination with seven organic solvents or mixtures of solvents (*n*-hexane, cyclohexane, acetone, toluene, 99:1 *n*-hexane / ethyl acetate, 90:10 *n*-hexane / acetone, and 50:50 *n*-hexane / DCM) were tested as additional clean-up. It was found that Strata SI-1 Silica 500 mg / 6 mL cartridges with cyclohexane as eluent provided optimal recovery rates for analytes and resulted in the necessary purity of the final extracts.

Analytical performance of the method

The developed method was validated using spiked fish homogenate and essential performance parameters such as linearity, limit of quantification (LOQ), accuracy (recovery), as well as intra- and inter-day repeatability were checked (Table 2). Five-point calibration curves over the concentration range for native DRCs from 1.00 to 100 pg μL^{-1} provided correlation coefficients (R^2) higher than 0.997, with the residual error of calibration below 10%. The instrumental LOQs (i-LOQs, expressed as the mass of analyte injected on column) achieved in this work ranging from ~10 femtograms for the most sensitive detection of Dec 603 to ~1 pg for the least sensitive detection of 1,3-DPMA corresponded to method LOQs (m-LOQs) in the range of 0.04 – 5.3 pg g^{-1} fresh weight (f.w.). The average recovery was calculated as the mean value over two days for each validation level and was found to be in the range from 70 to 120% for all compounds except Dec 604, for which elevated recovery values from 121 to 126% were observed. The method precision in terms of repeatability and reproducibility was below 10% for all target analytes, except 1,3-DPMA, which showed a slightly higher inter-day precision of 14.8%. The typical recoveries of $^{13}C_{10}$-labeled internal standards for selected DRCs provided by the developed method were in the range of 40–77 %.

Table 2.

Performance characteristics of the GC-HRMS method

Compound	i-LOQ, pg	m-LOQ, pg g^{-1} f.w.	Linearity, R^2	1st spiking level (25 pg g^{-1} f.w.)			2nd spiking level (50 pg g^{-1} f.w.)		
				Intra-day precision (n=2)	Inter-day precision (n=2)	Recovery (n=2)	Intra-day precision (n=2)	Inter-day precision (n=2)	Recovery (n=2)
DPMA	1.06	5.32	0.997	7.6	7.2	98	14.4	14.8	92
Mirex	0.01	0.07	0.998	7.6	7.3	70	4.9	6.4	73
Dec 602	0.03	0.17	0.999	2.9	2.8	99	1.7	1.9	102
DBHCTD	0.20	0.99	0.999	4.5	4.7	95	5.2	5.2	101
Dec 603	0.01	0.04	0.998	3.4	3.3	95	2.9	3.0	100
Dec 604	0.08	0.40	0.998	3.2	6.3	121	4.1	6.9	126
Cl10DP	0.01	0.04	0.999	4.7	5.5	85	3.8	4.1	89
***Syn*-DP**	0.02	0.12	0.999	1.8	1.8	104	1.8	2.0	108
Cl11DP	0.01	0.06	0.999	4.2	4.2	100	3.8	3.7	102
***Anti*-DP**	0.05	0.24	0.999	4.8	4.6	97	9.9	9.3	104

Application to real samples

The developed method was applied for testing of 15 food samples (Table 3). The number of samples analyzed within the framework of this study was small, and apart from confirming the presence of these compounds in the samples, any observations could be only indicative. 1,3-DPMA, Dec 604, and both products of DP dechlorination were not detected in any of the samples. The detection frequency (values provided in the parentheses) for the rest of the DRCs were in the following order: Mirex and *syn*-DP (87%) > *anti*-DP (80%) > Dec 602 (60%) > DBHCTD and Dec 603 (both 10%). Cod liver was the most contaminated matrix among the investigated food groups, showing the concentrations of ∑DRCs from 115 to 628 pg g^{-1} f.w. Despite the fact that Mirex is the most frequently detected analyte among the investigated compounds, its average contribution to the ∑DRCs constituted only 16% and 3% for dairy and meat foodstuffs, respectively. In the case of fish products, Mirex was confirmed to be a major contaminant, with the exception of one cod liver sample, where *anti*-DP was the major contributor. The total-DP isomer concentrations (sum of *syn*-DP and *anti*-DP) ranged from undetectable to 76.3 pg g^{-1} f.w., revealing strong *anti*-DP enrichment and *syn*-DP depletion for those samples where both of the DP isomers were detected. Dec 603 was confirmed in rabbit liver, herring and cod liver samples, while it was below the limit of quantification in dairy products. The alternative FR representative DBHCTD was detected only in sprats and cod liver samples, which was in line with our previous study on the analysis of DRCs in Baltic wild salmon [6] and indicates that the source of this compound could originate from the marine environment of the Baltic sea.

Table 3.

DRCs concentrations in food samples expressed in pg g^{-1} f.w.

Sample	Fat content, %	Mirex	Dec 602	DBHCTD	Dec 603	*Syn*-DP	*Anti*-DP	∑DRCs
milk	4.1	0.09	< LOQ	< LOQ	< LOQ	0.32	< LOQ	0.41
eggs	7.8	0.32	0.32	< LOQ	< LOQ	0.81	4.08	5.54
eggs	8.9	0.36	0.31	< LOQ	< LOQ	0.80	2.28	3.76
cheese	34.5	0.09	< LOQ	< LOQ	< LOQ	0.22	< LOQ	0.31
butter	87.3	0.24	< LOQ	< LOQ	< LOQ	0.88	< LOQ	1.11
chicken	18.5	< LOQ	0.18	< LOQ	< LOQ	< LOQ	1.10	1.28
chicken liver	6.0	0.12	< LOQ	< LOQ	< LOQ	0.57	2.31	3.00
pork liver	6.6	< LOQ	< LOQ	< LOQ	< LOQ	0.73	2.30	3.03
rabbit liver	5.9	0.91	1.31	< LOQ	0.70	3.08	15.5	21.5
sheep liver	4.0	0.42	< LOQ	< LOQ	< LOQ	0.34	4.56	5.32
sprat	17.7	9.03	2.96	4.31	< LOQ	1.62	5.74	23.7
herring	11.6	3.31	1.51	< LOQ	0.19	< LOQ	1.26	6.28
cod liver	84.2	88.1	19.3	5.76	< LOQ	0.40	1.43	115
cod liver	79.1	50.4	14.2	55.7	7.11	6.51	69.8	204
cod liver	47.3	324	136	131	3.10	5.02	29.5	628

References

[1] Shen, L.; Reiner, E. J.; Helm, P. A.; Marvin, C. H.; Hill, B.; Zhang, X.; MacPherson, K. A.; Kolic, T. M.; Tomy, G. T.; Brindle, I. D. Historic Trends of Dechloranes 602, 603, 604, Dechlorane Plus and Other Norbornene Derivatives and Their Bioaccumulation Potential in Lake Ontario. Environ. Sci. Technol. 2011, 45, 3333–3340.

[2] Sverko, E., Reiner, E.J., Tomy, G.T., Mccrindle, R., Shen, L., Arsenault, G., Zaruk, D., Macpherson, K.A., Marvin, C.H., Helm, P.A., McCarry, B.E., 2010. Compounds structurally related to Dechlorane Plus in sediment and biota from Lake Ontario (Canada). Environ. Sci. Technol. 44, 574–579.

[3] Sühring, R.; Byer, J.; Freese, M.; Pohlmann, J.-D.; Wolschke, H.; Möller, A.; Hodson, P. V.; Alaee, M.; Hanel, R.; Ebinghaus, R. Brominated flame retardants and Dechloranes in European and American eels from glass to silver life stages. Chemosphere 2014, 116, 104–111.

[4] Kim, J.; Son, M.-H.; Kim, J.; Suh, J.; Kang, Y.; Chang, Y.-S. Assessment of Dechlorane compounds in foodstuffs obtained from retail markets and estimates of dietary intake in Korean population. Journal of Hazardous Materials 2014, 275, 19–25.

[5] L'Homme, B.; Calaprice, C.; Calvano C. D.; Zambonin C.; Leardi, R.; Focant, J.-F. Ultra trace measurement of Dechloranes to investigate food as a route of human exposure. Chemosphere 2015, 139, 525–533.

[6] Rjabova, J.; Bartkevics, V.; Zacs, D. The occurrence of Dechlorane Plus and related norbornene-based flame retardants in Baltic wild salmon (Salmo salar). Chemosphere, 2016, 147, 210-217.