GERSTEL AppNote

Quantification of Persistent Organic Pollutants in Dietary Supplements Using Stir Bar Sorptive Extraction Coupled with GC-MS/MS and Isotope Dilution Mass Spectrometry

Weier Hao[1], H. M. 'Skip' Kingston[1], Ashley Dillard[1], John Stuff[2], Matt Pamuku[3]

1. Department of Chemistry and Biochemistry, Duquesne University, 600 Forbes Ave, Pittsburgh, PA 15282, United States.

2. GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090, United States

3. Applied Isotope Technologies, 851 Stella Court, Sunnyvale, CA 94087, United State

Keywords

Persistent organic pollutants; Dietary supplements; Stir bar sorptive extraction; GC-MS/MS; Isotope dilution mass spectrometry

Abstract

In this document we describe a method developed to quantify persistent organic pollutants (POPs) including polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) in dietary supplements using stir bar sorptive extraction (SBSE)-GC-MS/MS-isotope dilution mass spectrometry (IDMS). This method enables accurate, precise, and sensitive quantification of POPs in plant-extract based dietary supplement products commercially available in the United States. When compared with calibration curves, IDMS provided more accurate and precise measurements. The mean error of measurements using this method was 7.24% with a mean RSD of 8.26%. The application of GC-MS/MS enabled approximately two-order-of-magnitude lower limits of quantification compared with GC-MS. Twelve commercially available plant-extract based dietary supplement samples were analyzed using this method. PAHs including naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, chrysene, and benzo[a]pyrene were found in most of the products and had average concentrations over 1 ng/g. OCPs were detected less frequently than PAHs in these products, and none of the OCPs had mean concentrations over 1 ng/g. These results were compared with existing guidelines and none of the analytes in the samples were found to be above the daily allowable limits.

Introduction

Persistent organic pollutants (POPs) are highly stable organic chemicals that resist photolytic, biological, and chemical degradation. They persist in the environment, bioaccumulate through the food chain, and may adversely impact human health and the environment.[1] Over the past decades, POPs have been released to the environment primarily due to human activities. They are highly resistant to degradation, semi-volatile, hydrophobic, and toxic to living organisms. These characteristic properties of POPs turned them into one of the highest-priority environmental and human health concerns around the globe. POPs including PAHs and OCPs have been found in children's blood[2] and blood samples from a blood bank in Northern California.[3] Human's exposure to these toxins typically occurs through diet, drinking water, and air.[4] Dietary supplements are suspected to be one of the sources of the exposure. Dietary supplements are products intended to supplement the diet and typically contain dietary ingredients such as vitamins, minerals, herbs, fibers, botanical extracts, amino acids, and other substances that increase an individual's dietary intake.[5] More than half of adults in the US take dietary supplements daily or occasionally.[6] The majority of botanicals or herbals

that are used as raw materials of dietary supplements are farmed using conventional agricultural practices that may involve pesticide application.[7] In addition, POPs generated in different agricultural or industrial regions can be transported over long distances and contaminate the botanicals that are used in dietary supplement formulations. Thus, POPs in dietary supplements can be a source of xenobiotic toxins in the human body that can adversely impact human health. Dietary supplements from worldwide have been analyzed and found to contain POPs such as PAHs, polychlorinated biphenyls, polybrominated diphenyl ethers, and pesticides.[8-13] Physicians and nutritionists generally suggest taking dietary supplements to support metabolism and improve health without considering the toxins they may contain. Currently, no routine method exists for the extraction and quantitative analysis of POPs in dietary supplements. Therefore, the development of an effective and efficient extraction and analysis protocol for POPs in dietary supplements is important to assure product quality, public safety, and regulatory compliance.

Experimental

Instrumentation:

Agilent GC-MS/MS (7890B GC, 7010 MS/MS)
GERSTEL MultiPurpose Sampler (MPS robotic),
Thermal Desorption Unit (TDU 2) &
Cooled Injection System (CIS 6)

Analysis conditions

Column:	HP-5 MS (Agilent) di = 0.25 mm, df = 0.25µm, L = 30 m
Pneumatics:	He, Constant flow = 1.2 mL/min
Oven:	40 °C; 10 °C/min; 290 °C
TDU:	Splitless: 40 °C (0 min), 300 °C/min, 300 °C (15 min)
CIS:	Solvent vent (50 mL/min) -10 °C (15 min), 12°C/sec, 300° C (3 min)

	RT (min)	Precursor (Da)	Product (Da)		RT (min)	Precursor (Da)	Product (Da)	CE(V)
Naphthalene	8.964	128	127	Naphthalene-D8	8.923	136	134	15
Acenaphthene	13.024	152	151	Acenaphthene-D10	12.956	162	160	30
Fluorene	14.224	165	164	Fluorene-D10	14.156	175	173	30
Phenanthrene	16.449	178	177	Phenanthrene-D10	16.394	188	186	15
Fluoranthene	19.267	202	201	Fluoranthene-D10	19.227	212	210	5
Pyrene	19.780	202	201	Pyrene-D10	19.739	212	210	5
Benzo[a]anthracene	22.653	228	227	Benzo[a]anthracene-D12	22.612	240	238	5
Chrysene	22.747	228	227	Chrysene-D12	22.693	240	238	5
Benzo[b]fluoranthene	25.161	252	251	Benzo[b]fluoranthene-D12	25.106	264	262	5
Benzo[k]fluoranthene	25.228	252	251	Benzo[k]fluoranthene-D12	25.173	264	262	5
Benzo[a]pyrene	25.997	252	251	Benzo[a]pyrene-D12	25.942	264	262	5
Indeno[1,2,3-cd] pyrene	29.948	276	275	Indeno[1,2,3-cd]pyrene-D12	29.852	288	286	25
Benzo[ghi]perylene	31.041	276	275	Benzo[ghi]perylene-D12	30.918	288	286	25
α-HCH	15.531	181	145	α-HCH-^{13}C6	15.531	187	151	10
β-HCH	16.111	181	145	β-HCH-^{13}C6	16.111	187	151	10
γ-HCH	16.233	181	145	γ-HCH-^{13}C6	16.233	187	151	10
δ-HCH	16.732	181	145	δ-HCH-^{13}C6	16.732	187	151	10
DDE	20.304	246	176	DDE-^{13}C12	20.303	258	188	30
DDD	21.088	235	165	DDD-^{13}C12	21.087	247	177	20
DDT	21.776	235	165	DDT-13C12	21.775	247	177	20
Chlorpyrifos	18.518	314	258	Chlorpyrifos-D10	18.436	324	260	15

Table 1. GC-MS/MS method parameters of the natural analytes and their isotopes.

Sample Preparation

A sample of 1 g of plant-extract based dietary supplement in powder form was added into each vial and 2 mL of acetonitrile, along with 8 mL of deionized water was then added. The extraction was performed using 10 mm x 0.5 mm (length × film thickness) PDMS stir bars (GERSTEL Twister®) supplied by GERSTEL. The stirring process was carried out using a GERSTEL multiple-position magnetic stirring plate at a stirring rate of 1200 rpm. After stirring for one hour, the stir bar was taken out of the vial with tweezers, rinsed with deionized water, and carefully dried with clean wipe. Then the stir bar was placed in a desorption tube and the tube was loaded in a sample tray. Thereafter, the samples were handled by GERSTEL dual-head robotic MultiPurpose Sampler (MPS), which introduced the tubes sequentially into the Thermal Desorption Unit (TDU 2). A Cooled Injection System (GERSTEL CIS 6) PTV type GC inlet was used as the injector for the GC-MS/MS platform (7890B GC, 7010 MS/MS, Agilent Technologies, Santa Clara, CA).

Desorption temperature of the TDU was set at 290 ºC. The analytes were desorbed under helium in the TDU, transferred to the CIS and cryofocused at -10 ºC by liquid nitrogen for 15 min. The CIS was then heated at 12 ºC per second to 300 ºC to transfer the analytes to the GC column (HP-5 MS, Agilent, 30 m × 0.25 mm internal diameter, 0.25 µm film thickness, 5%-phenyl methyl siloxane). The carrier gas was helium, at a flow rate of 1.2 mL/min. The GC oven temperature was ramped at 10 ºC per min from 40 ºC to 290 ºC, and then held at 290 ºC. After electron ionization, the analytes were analyzed by the triple-quad mass analyzer. Identification and quantification of analytes was conducted using multiple reaction monitoring (MRM) mode. The MRM transitions of the analytes and the isotopes are shown in Table 1. Data analysis was performed using the Agilent MassHunter Workstation software.

Isotope Dilution Mass Spectrometry

Isotope dilution mass spectrometry (IDMS) is a quantification method which involves spiking the sample matrix with a known amount of isotope analogue of target analyte. The resulting isotope ratio is measured by mass spectrometry once the isotope spike is equilibrated with the sample matrix. By using this ratio, the concentration of the analyte in the sample is calculated. Specifically:

$$R_m = \frac{A_s C_s W_s + A_{sp} C_{sp} W_{sp}}{B_s C_s W_s + B_{sp} C_{sp} W_{sp}}$$

In this equation, R_m is the measured isotope ratio of A to B. A_s and B_s are fractions of A (target analyte) to B (the isotope analogue) in the sample, respectively. A_{sp} and Bsp are fractions of A and B in the spike, respectively. C_s is concentration of the target analyte in the sample and C_{sp} is concentration of the spike. W_s and W_{sp} are weights of the sample and the spike, respectively. In this equation, each term is known or can be determined by mass spectrometry except C_s. Therefore, the direct mathematical IDMS equation to calculate the concentration of the target analyte in the sample, C_s, is as follow:

$$C_m = \frac{C_{sp} W_{sp} (A_{sp} - R_m B_{sp})}{W_s (R_m B_s - A_s)}$$

IDMS is a direct quantification method entirely different from calibration curve methods. IDMS eliminates the need for serial dilutions and external calibrations. By spiking the sample with isotope analogues, each IDMS measurement becomes its own "calibration". Once the equilibrium between the sample and the spike is achieved, reproducible recoveries of the analytes are not required. Factors that will affect the recoveries and cause biases such as partial loss of the analytes, variable analyte recovery, interferences during analysis, and instrument performance drift are eliminated as these factors have the same effects on the analytes and their corresponding isotope analogues, and thus "Rm" is not influenced. In addition, IDMS methods are less time-consuming than calibration curve methods since the procedure to create calibration curves is not necessary for IDMS. Given that at least five standards with different concentrations are needed to create a calibration curve for each analyte, the time for analysis using IDMS is less than one sixth of the analysis time using calibration curves. Application of IDMS for analysis of organic compounds has demonstrated significant quantitative improvements by reducing errors and uncertainties.[14-15] Detailed description and discussion of IDMS can be found in the EPA Method 6800. In this work, IDMS was employed to quantify POPs in dietary supplements and the results were compared with data obtained using calibration curves.

The preparation of the labelled standards and spiking of the samples is described elsewhere. [3,13,14,15]

Results and Discussion

Optimization of the Extraction Procedure

Extraction solvents and time of the SBSE method were optimized using a set of recovery experiments. The unlabeled natural standards of the analytes were spiked into a plantextract based dietary supplement product to create a reference standard at a concentration of 10 ng/g. Relative recoveries of the analytes using different extraction procedures were compared to determine the optimized extraction solvents and time. The extraction time was set at 1 h to compare relative recoveries of different extraction solvents including: 1) 10 mL deionized water, 2) 2 mL methanol + 8 mL deionized water, 3) 5 mL methanol + 5 mL deionized water, 4) 2 mL acetonitrile + 8 mL deionized water, and 5) 5 mL acetonitrile + 5 mL deionized water. As shown in Figure 1, 2 mL acetonitrile + 8 mL deionized water had the highest overall recovery and showed higher recoveries for most of the analytes especially the ones with higher molecular weight. Using 2 mL acetonitrile and 8 mL deionized water as extraction solvents, different extraction time (30, 60, 90, and 120 min) were investigated. The relative recovery of most analytes reached equilibrium at 60 min.

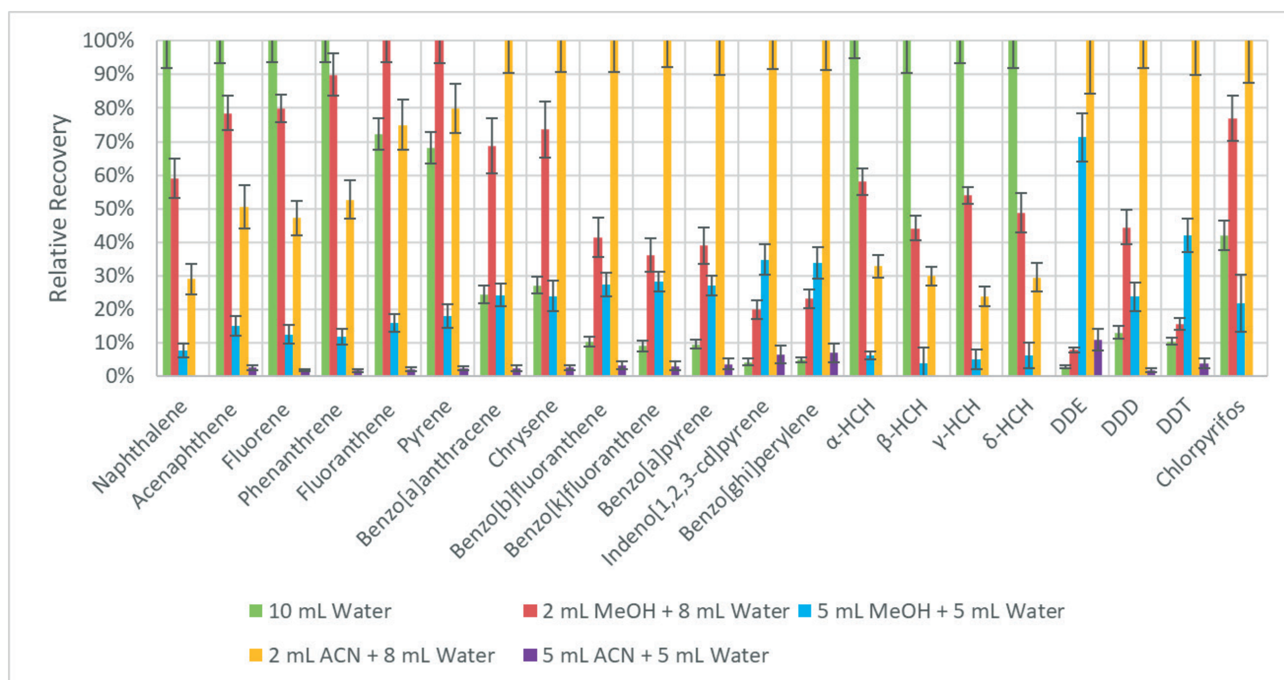

Figure 1. Relative recovery of the spiked analytes in dietary supplement sample using different extraction solvents (n=5, 95% CI). The relative recovery of each analyte was normalized to a 0-100% scale.

Method Validation

After optimizing the experimental parameters, the method was validated by quantifying POPs with known concentrations in blank-subtracted dietary supplement samples. Ideally, a dietary supplement standard reference material containing the analytes would be used for validation of the method. However, such a standard reference material is not currently available. A plant-extract based dietary supplement product in fine powder form was used to create the reference standards at four different concentrations ranging from 0.103 to 3.36 ng/g. These concentrations were shown as concentrations 1, 2, 3, and 4 in later discussions. These reference standards were spiked with isotopically enriched standards to quantify the natural analytes using IDMS. Five replicates were performed at each concentration level. Measurements of the spiked analytes in the reference standards were compared with the theoretical values at the four different concentrations. For most of the analytes (except acenaphthene), there was no statistical difference between the measured values and the theoretical values, which confirmed the accuracy of the method. For acenaphthene, a reverse-IDMS method was employed to verify and recalibrate the labelled concentration of the isotope and natural standards, which is discussed in detail in a peer-reviewed publication.13 Except for acenaphthene, the error of the measurements was in a range between 3.08% and 14.8%, whereas RSD ranged from 4.48% to 12.9%.

Comparison between IDMS and Calibration Curves

Measurements using the IDMS method were compared with measurements using calibration curves. Standard five-point matrix-matched calibration curves were created for each analyte. Isotope standards were added as internal standards (IS) to create calibration curves with IS for each analyte. Mean percent errors and RSDs of the measurements using IDMS, calibration curves, and calibration curves with IS are shown in Table 2.

		Concentration 1	Concentration 2	Concentration 3	Concentration 4
CC	Error (%)	61.6	39.8	32.4	26.4
	RSD (%)	30.0	16.6	10.2	12.1
CC with IS	Error (%)	41.6	9.46	11.8	8.52
	RSD (%)	20.5	7.74	6.64	5.83
IDMS	Error (%)	10.1	6.98	5.89	6.02
	RSD (%)	13.9	7.86	6.69	4.56

Table 2. Mean percent error and RSD of the measurements of the spiked analytes in the dietary supplement samples at four different spiking concentrations using IDMS, calibration curves, and calibration curves with IS for quantification (n=5). Concentrations 1-4 are 0.103, 0.333, 1.13, and 3.36 ng/g, respectively.

Concentrations 1-4 are 0.103, 0.333, 1.13, and 3.36 ng/g, respectively. At concentration 1, the mean error of the measurements using calibration curves was greater than 60%. For naphthalene, α-HCH, and γ-HCH errors were over 100%. By adding IS the mean error of measurements at concentration 1 decreased to 41.6%. As a comparison, the mean error of measurements using IDMS was 10.1%. At concentrations 2-4, the mean error of the measurements decreased for all the three methods. Nevertheless, IDMS and calibration curves with IS had significantly lower mean error than calibration curves. Generally, the RSDs of the measurements decreased from lower to higher measured concentrations for all three methods. At concentration 1, the mean RSDs of the measurements using IDMS were significantly lower than calibration curves and calibration curves with IS. At concentrations 2-4, IDMS and calibration curves with IS had comparable results. A graphic comparison of the measurements using IDMS, calibration curves, and calibration curves with IS is shown in Figure 2. Phenanthrene was selected as an example to present the results. IDMS had observable advantages in accuracy and precision compared with calibration curves especially at the two lower measured concentrations.

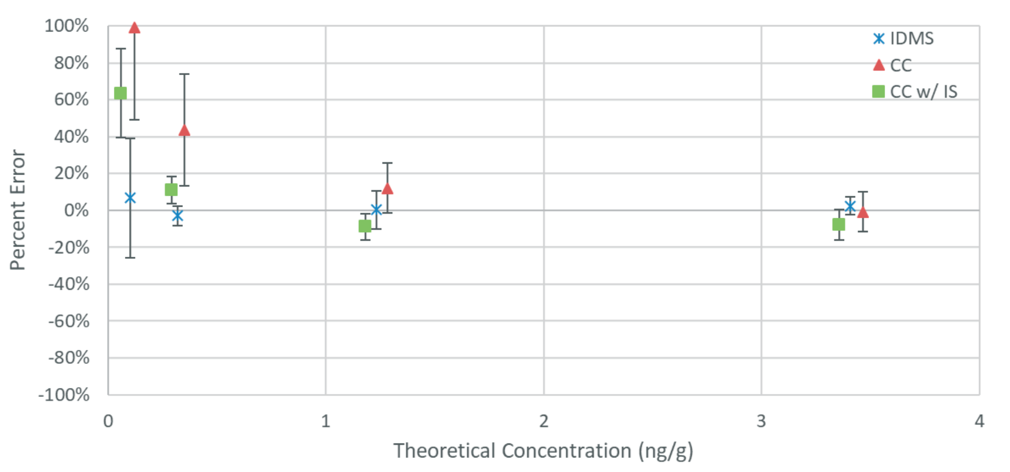

Figure 2. Comparing percent error of measurements of spiked phenanthrene in dietary supplement samples at the four different spiking concentrations using IDMS, calibration curves, and calibration curves with IS for quantification (n=5, 95% CI).

Comparison between GC-MS/MS and GC-MS

For comparison, the analytes were also quantified using SBSE-GC-MS-IDMS. The GC-MS instrument employed was 7890B GC, 5975C MS by Agilent Technologies (Santa Clara, CA). The stir bars, the autosamplers, the TDU and CIS system, and the experimental parameters were the same as employed in the GC-MS/MS method. The LOQs of the analytes using the GC-MS method ranged from 9.26 ng/g (δ-HCH) to 0.457 ng/g (pyrene). Compared GC-MS/MS results, the LOQs using GC-MS were approximately two orders of magnitude higher. In analysis of POPs at low concentrations, the improvements in LOQ using GC-MS/MS are crucial. The SBSE-GC-MS-IDMS method was used to analyze the spiked analytes in the dietary supplement reference standards at concentrations 1-4 which were mentioned in the earlier discussions. At concentrations 1-3, the majority of the measurements were not applicable since most of the spiking concentrations were below the LOQs. At concentration 4, the errors of measurements ranged from 1.44% to 16.2% with a mean value of 8.20%. The RSDs were in a range of 5.84-14.2% with a mean value of 9.56%. These errors and RSDs were mostly higher than using GC-MS/MS at the same concentration. This work demonstrated that the use of GC-MS/MS improved accuracy and precision of measurements at low concentration levels.

Analysis of Real Samples

After development, optimization, and validation, the method was used to analyze plant-extract based dietary supplement products that are commercially available in the US. Twelve products from seven different brands were selected for analysis. These products were deidentified and referred to as sample 1-12. Information of each product is listed in Table 3.

Sample number	Brand	Form	Simplified product description
1	A	Tablet	Multivitamin for energy and metabolism with vegetable ingredients
2	A	Tablet	Multivitamin for pregnant women with vegetable ingredients
3	A	Tablet	Multivitamin for energy and metabolism with vegetable ingredients
4	A	Tablet	Multivitamin for pregnant women with vegetable ingredients
5	B	Tablet	Multivitamin for pregnant women with vegetable and fruit ingredients
6	B	Tablet	Multivitamin for pregnant women with vegetable and fruit ingredients
7	B	Tablet	Multivitamin for pregnant women with vegetable and fruit ingredients
8	C	Tablet	Herbal based multivitamin for women
9	D	Powder	Herbal supplement for prostate health
10	E	Powder	Herbal supplement for urinary tract health
11	F	Powder	Herbal supplement for urinary tract health
12	G	Powder	Herbal supplement for female health

Table 3. Deidentified sample number, brand, form, and simplified description of the 12 dietary supplement samples analyzed.

The method discussed in the method validation section was used for quantification. Quantification results of the samples are shown in Figure 3. PAHs with lower molecular weight were detected frequently in these samples. DDT was the most frequently detected OCP. Eight samples were found to contain DDT. The PAHs were found at a higher concentration level. Naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, chrysene, and benzo[a]pyrene had average concentrations over 1 ng/g in the tested samples. On average, approximately 12 analytes were detected in each sample. A total of 16 analytes were detected in sample No. 5, which was the highest number detected. Eight were detected in sample No. 4, which was the lowest number detected. Sample No. 1-4 and 6-7 had mean toxin concentrations below 1 ng/g, whereas the rest of the samples had above-1 ng/g mean toxin concentrations. Sample No. 12 had a mean toxin concentration of 3.20 ng/g, which was the highest among all the samples. Based on the serving instruction of each dietary supplement product, the concentration of each quantified analyte was converted to daily intake amount (ng/day) and daily intake amount per body weight (ng/kg/day). The guidelines for some of the analytes from the Agency for Toxic Substances and Disease Registry (ATSDR) and the California Office of Environmental Health Hazard Assessment (OEHHA) Proposition 65 were listed in Table 4.

	ATSDR-Minimal Risk Level (mg/kg/day)	OEHHA Proposition 65- No Significant Risk Level (µg/day)
Naphthalene	0.6	5.8
Acenaphthene	0.6	N/A
Fluorene	0.4	N/A
Phenanthrene	N/A	N/A
Fluoranthene	0.4	N/A
Pyrene	N/A	N/A
Benz[a]anthracene	N/A	0.033
Chrysene	N/A	N/A
Benzo[b]fluoranthene	N/A	0.096
Benzo[k]fluoranthene	N/A	N/A
Benzo[a]pyrene	N/A	0.06
Indeno[1,2,3-cd]pyrene	N/A	N/A
Benzo[ghi]perylene	N/A	N/A
α-HCH	N/A	0.3
β-HCH	N/A	0.5
γ-HCH	N/A	0.6
δ-HCH	N/A	N/A
DDE	N/A	2 (DDE, DDD, and DDT combined)
DDD	N/A	2 (DDE, DDD, and DDT combined)
DDT	0.00005	2 (DDE, DDD, and DDT combined)
Chlorpyrifos	0.001	0.0001 (mg/kg/day; child-specific refence dose)

Table 4. Minimal risk levels set by ATSDR and no significant risk levels in the Proposition 65 of OEHHA for the analytes. Analytes without specific guidelines are shown as N/A.

Comparing the quantification results with these guide-lines, none of the samples had analytes that exceeded the daily allowable levels. However, for sample No.8, the daily intake amount of benzo[a]pyrene was 30.8 ng/day, which approached approximately half of the no significant risk level set by the OEHHA Proposition 65. For sample No. 5 and 12, the daily intake amount of benz[a]anthracene was 3.52 and 5.02 ng/day, respectively. Both of these results were between 10-20% of the no significant risk level set by the OEHHA under Proposition 65 regulations, which is 33 ng/day. For the rest of the analytes in the 12 tested dietary supplement samples, the daily intake amounts were generally within 10% of the daily allowable levels.

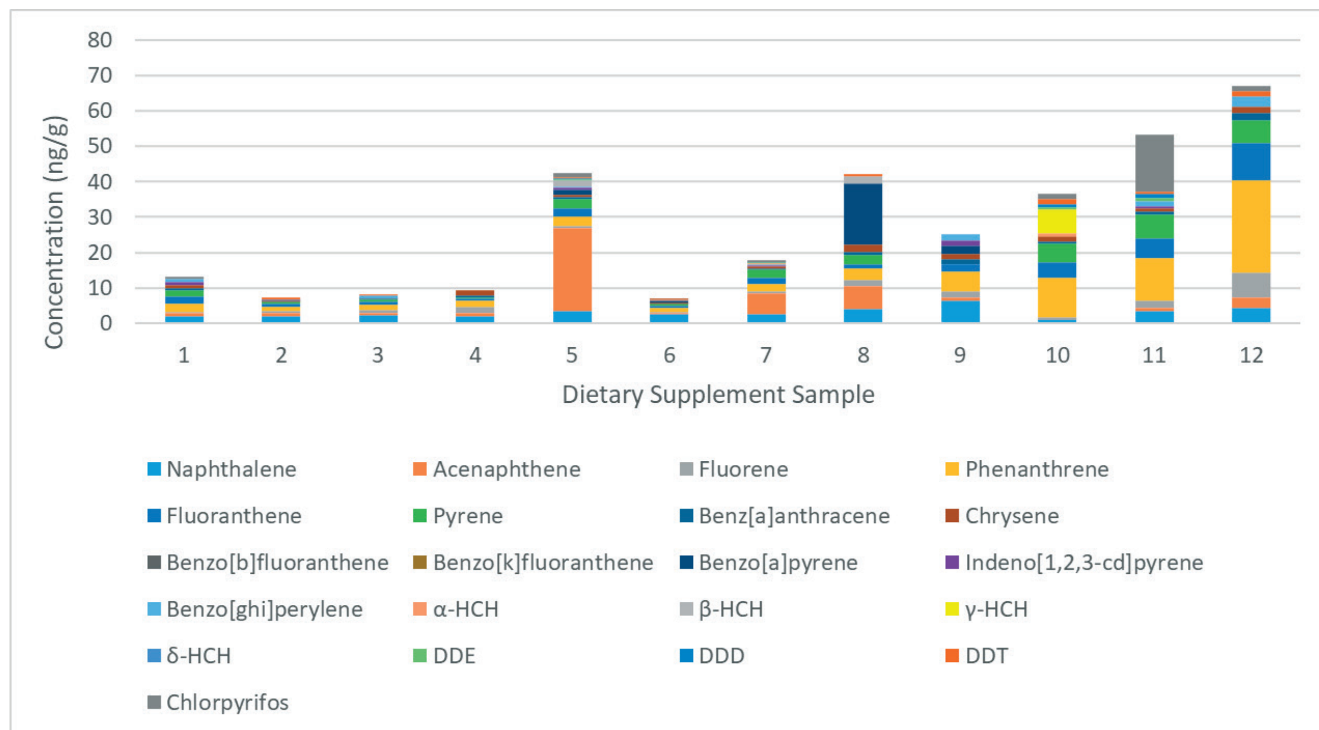

Figure 3. Measurements of the analytes in the 12 commercially available plant-extract based dietary supplement samples using SBSE-GC-MS/MS-IDMS (n=5).

Conclusions

This study elucidated the development of an accurate, precise, sensitive, and efficient quantification method for POPs in plant-extract based dietary supplements. IDMS was compared with calibration curves and was demonstrated to have advantages in terms of accuracy, precision, and efficiency of the analysis. Compared with GC-MS, the GC-MS/MS was able to perform analysis at lower LOQs. The optimized and validated method was used to quantify POPs in 12 commercially available plant-extract based dietary supplements sold in the US. PAHs such as naphthalene, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene, chrysene, and benzo[a]pyrene were detected in most of the products. DDT was the most frequently detected OCP and was found in 8 products. On average, 12 analytes were detected in each sample with a mean concentration of 1.31 ng/g. These measurements were converted to daily intake amount and compared with the existing guidelines. None of the quantified analytes in the investigated dietary supplement products exceeded the thresholds set for individual toxins. Symbiotic adverse effect from multiple POPs in dietary supplements remain an ongoing concern, especially for pregnant women and children.

References

(1) Stockholm Convention - What are POPs. http://chm.pops.int/TheConvention/ThePOPs/tabid/673/Default.aspx.

(2) Boggess, A.; Faber, S.; Kern, J.; Kingston, H. S., Mean serum-level of common organic pollutants is predictive of behavioral severity in children with autism spectrum disorders. Scientific Reports **2016**, 6, 26185.

(3) Hao, W.; Kingston, H. M. S.; Dillard, A.; Macherone, A.; Stuff, J.; Pamuku, M., Quantification of Persistent Organic Pollutants in Human Whole Blood Samples Using Stir Bar Sorptive Extraction Coupled with GC/MS/MS and Isotope Dilution Mass Spectrometry. Microchemical Journal **2020**, 153, 104279.

(4) Könemann, W.; Pieters, M., Confusion of concepts in mixture toxicology. Food and Chemical Toxicology **1996**, 34 (11-12), 1025-1031.

(5) National Institute of Health- Office of Dietary Supplement. http://ods.od.nih.gov/factsheets/DietarySupplements-HealthProfessional/.

(6) Tancell, P.; Rhead, M.; Trier, C.; Bell, M.; Fussey, D., The sources of benzo [a] pyrene in diesel exhaust emissions. Science of the Total Environment **1995**, 162 (2-3), 179-186.

(7) Chen, Y.; Al-Taher, F.; Juskelis, R.; Wong, J. W.; Zhang, K.; Hayward, D. G.; Zweigenbaum, J.; Stevens, J.; Cappozzo, J., Multiresidue Pesticide Analysis of Dried Botanical Dietary Supplements Using an Automated Dispersive SPE Cleanup for QuEChERS and High-Performance Liquid Chromatography–Tandem Mass Spectrometry. Journal of agricultural and food chemistry **2012**, 60 (40), 9991-9999.

(8) Rawn, D. F.; Breakell, K.; Verigin, V.; Nicolidakis, H.; Sit, D.; Feeley, M., Persistent organic pollutants in fish oil supplements on the Canadian market: polychlorinated biphenyls and organochlorine insecticides. Journal of food science **2009**, 74 (1), T14-9.

(9) Marti, M.; Ortiz, X.; Gasser, M.; Marti, R.; Montana, M. J.; Diaz-Ferrero, J., Persistent organic pollutants (PCDD/Fs, dioxin-like PCBs, marker PCBs, and PBDEs) in health supplements on the Spanish market. Chemosphere **2010**, 78 (10), 1256-62.

(10) Ruiz-Delgado, A.; Martínez-Domínguez, G.; Romero-González, R.; López-Ruiz, R.; Frenich, A. G., Determination of polycyclic aromatic hydrocarbons in soy isoflavone nutraceutical products by gas chromatography coupled to triple quadrupole tandem mass spectrometry. Journal of separation science **2016**, 39 (3), 528-536.

(11) Chen, Y.; Lopez, S.; Hayward, D. G.; Park, H. Y.; Wong, J. W.; Kim, S. S.; Wan, J.; Reddy, R. M.; Quinn, D. J.; Steiniger, D., Determination of multiresidue pesticides in botanical dietary supplements using gas chromatography–triple-quadrupole mass spectrometry (GC-MS/MS). J Agr Food Chem **2016**, 64 (31), 6125-6132.

(12) Szarka, A.; Turková, D.; Hrouzková, S., Dispersive liquid-liquid microextraction followed by gas chromatography–mass spectrometry for the determination of pesticide residues in nutraceutical drops. Journal of Chromatography A **2018**, 1570, 126-134.

(13) Hao, W.; Kingston, H. S.; Dillard, A.; Stuff, J.; Pamuku, M., Quantification of persistent organic pollutants in dietary supplements using stir bar sorptive extraction coupled with GC-MS/MS and isotope dilution mass spectrometry. Food Additives & Contaminants: Part A **2020**, 1-14.

(14) Hao, W.; Kingston, H. M. S.; Dillard, A.; Macherone, A.; Stuff, J.; Pamuku, M., Quantification of Persistent Organic Pollutants in Human Whole Blood Samples Using Stir Bar Sorptive Extraction Coupled with GC/MS/MS and Isotope Dilution Mass Spectrometry. Microchemical Journal **2020**.

(15) Boggess, A. J.; Rahman, G. M.; Pamukcu, M.; Faber, S.; Kingston, H. S., An accurate and transferable protocol for reproducible quantification of organic pollutants in human serum using direct isotope dilution mass spectrometry. Analyst **2014**, 139 (23), 6223-6231.

GERSTEL AppNote

GERSTEL GmbH & Co. KG
Eberhard-Gerstel-Platz 1
45473 Mülheim an der Ruhr
Germany
+49 (0) 208 - 7 65 03-0
+49 (0) 208 - 7 65 03 33
gerstel@gerstel.com
www.gerstel.com

GERSTEL Worldwide

GERSTEL, Inc.
701 Digital Drive, Suite J
Linthicum, MD 21090
USA
+1 (410) 247 5885
+1 (410) 247 5887
sales@gerstelus.com
www.gerstelus.com

GERSTEL AG
Wassergrabe 27
CH-6210 Sursee
Switzerland
+41 (41) 9 21 97 23
+41 (41) 9 21 97 25
swiss@ch.gerstel.com
www.gerstel.ch

GERSTEL K.K.
1-3-1 Nakane, Meguro-ku
Tokyo 152-0031
SMBC Toritsudai Ekimae Bldg 4F
Japan
+81 3 5731 5321
+81 3 5731 5322
info@gerstel.co.jp
www.gerstel.co.jp

GERSTEL LLP
10 Science Park Road
#02-18 The Alpha
Singapore 117684
+65 6779 0933
+65 6779 0938
SEA@gerstel.com
www.gerstel.com

GERSTEL (Shanghai) Co. Ltd
Room 206, 2F, Bldg.56
No.1000, Jinhai Road,
Pudong District
Shanghai 201206
+86 21 50 93 30 57
china@gerstel.com
www.gerstel.cn

GERSTEL Brasil
Av. Pascoal da Rocha Falcão, 367
04785-000 São Paulo - SP Brasil
+55 (11)5665-8931
+55 (11)5666-9084
gerstel-brasil@gerstel.com
www.gerstel.com.br

Information, descriptions and specifications in this Publication are subject to change without notice. GERSTEL, GRAPHPACK and TWISTER are registered trademarks of GERSTEL GmbH & Co. KG.

© Copyright by GERSTEL GmbH & Co. KG

Awarded for the active pursuit of environmental sustainability