

Validation of a GC-MS/MS method for determination of organochlorine pesticides in fish products using ethyl acetate extraction

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Abstract

Organochlorine pesticides (OCPs) have been extensively used in agriculture due to their biocide activity. Though their use has been banned since many years before, because of their persistence and tendency for bioaccumulation, they often are detected and show presence in plant, animal and environment samples. A lot of studies, research and monitoring have been carried out from the respective scientific institutions and government in Albania, and the data obtained have confirmed the continuous presence of OCP and what is more they show the potential risk that our consumers run by consuming plant or/and animal origin food products. This continuous monitoring process requires not only hard work, efforts and time but reliable and consistent results as well, considering the fact of the low concentration level and difficult matrixes where these compounds are often encountered. In this paper we have presented the validation process and the respective results obtained using a GC MS/MS

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method for OCP determination in fish samples. The method is already in use in the Laboratory of Residues in Animal Origin Products in Food Safety and Veterinary Institute in Albania which is the only responsible body for National Residues Monitoring Plan in our country. The results of the validation process show that this method complies with the requirements of SANTE/11813/2017 guide.

Keywords: POPs, DTT, validation, GC MS/MS, environmental pollution, fish.

AIMS AND BACKGROUND

Several studies has shown (Mimoza Mukaj 2017, Aurel Nuro 2018, Elda Marku 2019) that though the production and use of organochlorine insecticides has been banned in Albania from early '90, they are constantly present in our environment. The goal of this paper is to show the results of the analytical performance validation of a method already used in our laboratory. We aimed at obtaining reliable results and decrease the total lab work required for the analyses of organochlorine pesticides (OCP) by Triple quadrupole GC-MS/MS in fish sample matrices but complying to EU regulatory guidance specifications for analytical performance as well. The method for the analysis of 17 OCP compounds is based on relatively simple sample preparation using ethyl acetate extraction followed by running into GC-MS/MS was successfully developed demonstrating acceptable precision, excellent linearity with R² values >0.995.

EXPERIMENTAL CONDITION

Sampling and Sample Preparation

For the validation of the presented GC-MS/MS method we have used shrimps as sample. The sample has been collected in local market. After collecting the sample have been stored and transported in lab in frozen condition. One kilogram of shrimp sample was chopped and later homogenized using an Ultra-Turrax IKA-T50. 5g of sample was accurate weighted in 50 ml polypropylene tubes. 0.20 mg of PSA (primary secondary amine) and C18 sorbent was added in each

sample for the clean-up and 10 ml of ethyl acetate was used for the extraction. The mixture was homogenized by 20 minutes shaking as the first extraction step. A second extraction step was performed for another 10 minutes shaking after 5 g of sodium sulfate was added into the sample. The organic phase was separated by centrifugation and an aliquot of 1 ml of the organic extract phase was transferred in 2 ml autosample vial followed by injection into GC-MS/MS.

Method Set Up

Agilent 7890A GC system coupled to an Agilent 7000C Series Triple Quadrupole using EI interphase was used. The chromatographic column used was a HP-5MS capillary column (30 m, 0.25 mm id, 0.25 μ m film thickness). Oven operating condition was initial 50°C hold 0.6 min, first ramp 15°C/min to 180°C hold 1min, second ramp 7°C/min to 230°C followed by the last ramp 3 °C/min to 280°C. Quantifications were performed by using matrix matched calibration curves, from 6 to 200 μ g/kg. The data were acquired with Agilent Mass-Hunter Acquisition Software (B.07.02). Data analysis was performed with Agilent Mass-Hunter Quantitative Analysis Software (B.08.00).

RESULTS AND DISCUSSION

Method Performance

The following organochlorine compounds shown in the table 1 were used to validate the method. Their extraction was performed using ethyl acetate, which has been shown as previously used in determination of pesticides residues in product of plant and animal origin (Pihlström T 2007, Despo Louc 2015). We used PSA and C₁₈ for the extract clean-up.

Table 1: Multiple reaction monitoring (MRM) transitions, collision energy (CE) for each transition and calibration curve equation during calibration

Nr.1	Analyte	Quantifier m/z	CE 1	Qualifier m/z	CE 2
1	Aldrin	298 -> 263	8	263 -> 193	30
2	α-BHC	181-> 145	15	181 -> 109	30
3	δ-BHC	181-> 145	15	181 -> 109	30
4	DDD, o,p'	235 -> 199.1	15	237 -> 165	20
5	DDD, p,p'	235 -> 199.1	15	237 -> 165	20
6	DDE, o,p'	246 -> 211	20	246 -> 176	30
7	DDE, p,p'	246 -> 211	20	246 -> 176	30
8	DDT, o,p'	235 -> 199.1	20	235 -> 165	20
9	DDT, p,p'	235 -> 199.1	20	237 -> 165	20
10	Dieldrin	263 -> 193	30	263 -> 191	30
11	Endrin	263 -> 193	35	263 -> 191	35
12	Heptachlor	274 -> 239	20	271.9 -> 236.8	25
13	Hexachlorobenzene	283.9 -> 248.8	25	283.9 -> 213.9	35
14	Lindane	218.8 -> 183	5	180.9 -> 145	12
15	Methoxychlor	227 -> 169	28	227 -> 141.1	40
16	Mirex	272 -> 237	20	272 -> 235	25

Calibration Curves and Linearity

Calibration curves were produced in the range of 6 µg/kg to 200 µg/kg for all the OCPs corresponding matrix extract at 5 concentration levels: 6, 10, 20, 100, and 200 ng/kg. All curves had a regression coefficient higher than 0.995.

At the lowest calibrated level (6 µg/L or 6 pg on column), the signal corresponds to the limit of detection (LOD). The 5 levels calibration curve of hexachlorobenzene and the ion transition is plotted in fig.1.

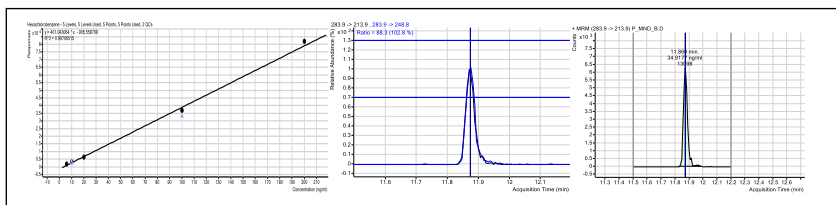


Figure 1: HCH calibration curve and ion transition

Matrix Effects

Matrix effects were assessed at the present method validation stage. The matrix effect was Calculated based on slopes of calibration curves following the above formula.

$$\%ME = (\text{Slope of Matric matched calibration curve/Slope Solvent curve}) * 100.$$

Table 2: Comparison of the response arising from solvent standards and from matrix-matched standards

Nr.	Compound name	Calibration formula in shrimp extract	Calibration formula in solvent	Matrix effect
1	Aldrin	$y = 215.151417 * x - 16.628015$	$y = 205.126100 * x - 361.008323$	105%
2	α - BHC	$y = 1464.472989 * x - 1788.218914$	$y = 1457.035011 * x - 3438.211985$	101%
3	δ - BHC	$y = 632.960006 * x - 212.659072$	$y = 612.151314 * x - 618.597948$	103%
4	DDD, o,p'	$y = 1094.224682 * x - 654.000512$	$y = 1127.150188 * x - 2269.121445$	97%
5	DDD, p,p	$y = 2839.480392 * x - 888.843970$	$y = 2553.955108 * x - 4314.149825$	111%
6	DDE, o,p'	$y = 6538.678956 * x - 1409.654570$	$y = 5767.833026 * x - 11524.776879$	113%
8	DDE, p,p'	$y = 358.094302 * x - 593.244869$	$y = 337.924631 * x - 732.639299$	106%
9	DDT, p,p'	$y = 7398.359898 * x - 20098.018392$	$y = 6496.433485 * x - 11412.960741$	114%
10	DDT-o,p'	$y = 1205.354922 * x - 1071.533575$	$y = 924.391037 * x - 4020.690983$	130%
11	Dieldrin	$y = 161.144370 * x + 116.217727$	$y = 153.757575 * x - 211.276137$	105%
12	Endrin	$y = 693.827455 * x - 155.775868$	$y = 663.693790 * x - 1792.172153$	105%
13	Heptachlor	$y = 491.807747 * x - 147.580672$	$y = 480.759511 * x - 1062.290258$	102%
14	Hexachlorobenzene	$y = 146.923571 * x - 11.631454$	$y = 140.043338 * x - 307.111738$	105%
15	Lindane	$y = 1468.593287 * x - 361.270162$	$y = 1482.101565 * x - 3561.676508$	99%
16	Methoxychlor	$y = 1889.459096 * x - 3150.461562$	$y = 1531.783044 * x - 6140.034561$	123%
17	Mirex	$y = 1072.876449 * x - 348.413569$	$y = 1007.477194 * x - 2357.925324$	106%

A maximum 20% difference is considered as acceptable according to Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. (SANTE/11813/2017 2017). As seen from the results in Table 2 no matrix effect is observed with one exception (DDTo,p`).

Precision in Spiked Matrix Samples

Recovery was studied by spiking control samples at two levels (10 and 50 $\mu\text{g}/\text{kg}$) of five replicates. In the tested matrixes (shrimp sample), the repeatability was established by performing analysis of spiked shrimp extracts fortified at two levels at 10 $\mu\text{g}/\text{kg}$ and 50 $\mu\text{g}/\text{kg}$. Five replicates were used for each spiking level, and the RSD was then calculated. The analytical performance parameters for all the OCP tested using GC-MS/MS system are shown in Table 3.

Table 3: Average recoveries (n = 5), standard deviations (SD) and RSD for samples fortified at 10 and 50 µg/kg.

Nr.	Analyte	10 ng/kg			50 ng/kg			Overall		
		Average recovery	SDV	RSD	Average recovery	SDV	RSD	Average recovery	SDV	RSD
1	Aldrin	85.69	9.80	11%	93.36	10.00	11%	87.48	10.22	12%
2	α-BHC	88.22	9.28	11%	92.25	5.10	6%	89.05	9.15	10%
3	β-BHC	98.07	18.82	19%	87.13	7.31	8%	96.04	17.69	18%
4	DDD, o,p'	82.28	11.92	14%	93.96	13.11	14%	84.84	12.94	15%
5	DDD, p,p'	95.19	15.64	16%	74.60	2.55	3%	93.67	16.01	17%
6	DDE, o,p'	91.81	8.90	10%	97.85	10.82	11%	93.32	9.58	10%
8	DDE, p,p'	90.89	11.29	12%	95.99	9.69	10%	92.01	11.02	12%
9	DDT, p,p'	87.41	14.25	16%	94.92	20.26	21%	88.66	15.15	17%
10	DDT, o,p'	89.58	11.79	13%	86.22	5.42	6%	89.33	11.40	13%
11	Dieldrin	81.60	16.20	20%	93.41	7.52	8%	83.70	15.60	19%
12	Endrin	87.30	9.35	11%	95.17	11.85	12%	89.69	10.57	12%
13	Heptachlor	96.07	16.59	17%	93.99	7.94	8%	95.70	15.31	16%
14	Hexachlorobenzene	87.56	9.84	11%	98.67	12.10	12%	88.99	10.62	12%
15	Lindane	83.67	11.26	13%	96.78	4.00	4%	85.36	11.46	13%
16	Methoxychlor	98.61	17.49	18%	93.05	21.42	23%	97.68	17.62	18%
17	Mirex	86.26	14.08	16%	90.37	13.26	15%	87.11	13.82	16%

Accepted accuracy values specified by SANTE document (SANTE/11813/2017), are recoveries included in the range between 70-120%. The precision is expressed by the values of the relative standard deviation of the measurements, and it is generally accepted with values $\leq 20\%$.

For the measurement of the concentration of the analyses and hence the calculation of the recovery the technique of direct comparison with a standard solution was used, in accordance with the requirements of SANTE document for pesticide residues.

CONCLUSION

Agilent 7890A GC system coupled to an Agilent 7000C Series Triple Quadrupole enabled simple method development, validation for all 17 selected OCP in selected matrixes. Quantitative performance of the system and methodology was excellent with a good level of linearity, high precision and acceptable recovery in the selected sample type.

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