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Development of a Rapid and Sensitive Routine Method of Analyses for Organochlorine Compounds in Fish: A Metrological Approach

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Abstract

The determination of organochlorine compounds in biological samples is very important, since these compounds are lipophilic and persistent in the environment and tend to accumulate in living organisms. However, considering the importance to apply adequate statistical tools to develop and evaluate analytical methods, this paper has as objective the optimization, validation and uncertainty evaluation of a rapid and sensitive routine method for analyses of organochlorine compounds in fish. For optimization of the method, statistical tools such as design of experiments were used, while guidance from SANCO 12571 and Eurachem/Citac Guide were used for the validation of the method and uncertainty estimation of the results respectively. Finally, the method was evaluated using a certified reference material (CRM) in an accredited laboratory that is in accordance with ISO/IEC 17025:2005, completing a suitable metrological approach ensuring that the method is fit for purpose.

Keywords: Organochlorine compounds; Validation; Uncertainty; ISO/IEC 17025; QuEChERS

Introduction

The indiscriminate and inappropriate use of pesticides have resulted in serious ecological problems, due to their contribution in the destruction of beneficial insects, water pollution, poisoning of animals, and contamination of food and population. Among the various types of pesticides, organochlorine compounds (OCs) have gained special attention. OCs are persistent and the presence of large amounts of these products can eventually contaminate rivers and lakes, while subsequently gradually increasing the contamination of the tissues (specially fat) in fish and other aquatic animals over time [1]. The determination of OCs in biological samples is very important, since these compounds are lipophilic and persistent in the environment and tend to accumulate in living organisms. OCs can cause harmful effects to human and aquatic life; chronic exposure over long periods of time to these compounds correlates with severe damage of high trophic level organisms, such as predators of fish, birds that feed on fish, and mammals. Ingestion is the major source of human exposure to these compounds [2]. In addition, polychlorinated biphenyls (PCBs) and some organochlorine pesticides are among compounds that are categorized as endocrine disrupters [3-4].

Fish is an important food commodity in the global trade market and knowing its risk to contamination by OCs it is mandatory to develop analytical methods that can determine the presence of these compounds in accordance with international safety regulations. The analytical methods that have been applied in determination of OCs in fish are liquid–liquid extraction (LLE), Sohxlet extraction, Accelerated Solvent Extraction (ASE), Matrix Solid Phase Dispersion (MSPD) [5-8]. Considering that fish`s tissue is a very complex matrix, the extraction methods needs a clean-up step, and at the same time the method needs to be fast. A good alternative is the application of QuEChERS, a method that is quick, easy, cheap, effective, rugged and safe [9].

There are some papers describing the development of QuEChERS method to analyse pesticides in fish tissue, but a few of them confirmed the method using certified reference materials (CRMs) and none of them evaluated the uncertainty of the analytical result [10-13]. The use of CRMs is an important key to method validation. It confirms that the method is capable of analyzing the compounds and it gives an exact and precise result. It is also essential that the development of an analytical method has to be done in a laboratory that operates with a quality system management to achieve reliability and traceability of the results.

It should be noted that official analysis must be performed by accredited laboratories in accordance with ISO/IEC 17025:2005. This practice ensures that the results have traceability and guaranteed reliability. In relation to global trade, these tests` results can be accepted by regulatory agencies without confirmatory analysis in countries that have mutual recognition agreement with the International Laboratory Accreditation Cooperation (ILAC).

This paper has as an objective the optimization, validation and uncertainty evaluation of a rapid and sensitive method for analysis of OCs in fish, developed in an accredited laboratory that is in accordance with ISO/IEC 17025.

Materials and Methods

Standards

The 17 reference compounds (Aldrin "ALD", c-Chlordane "cCLD", t-Chlordane "tCLD", p,p-DDD "ppD", p,p-DDE "ppE", o,p-DDT "opT", p,p-DDT "ppT", Dieldrin "DLD", Lindane "LIN", Hexachlorobenzene "HCB", Heptachlor Epoxide "HPX", PCB 101, 118, 138, 153, 180 and Mirex "MRX") were purchased from Accustandard (New Haven,

USA). Certified Reference Material NIST 1946 was purchased from National Institute of Standards & Technology (Gaithersburg, USA). Stock solutions were prepared in n-hexane at a concentration of the maximum residues limits (MRL) for each compound. Working solutions were stored at 4°C.

Chemicals

Solvent n-hexane HPLC-grade were purchased from Merck (Darmstadt, Germany) and sodium chloride, magnesium sulphate, primary secondary amine (PSA) and C18 were purchased from Sigma-Aldrich (St. Louis, USA).

Samples

Trouts were attained from an organic producer and were dead, without scales and vicera, refrigerated (-20°C), and were transported to Lanagro-SP (National Agricultural Laboratories of Ministry of Agriculture, Livestock and Supply).

Extraction

Samples of fish were removed from the freezer and left out for 30 min. Musculature was cut in small pieces and 2.000 ± 0.002 g were weighed in a 50 mL Falcon® tube. If the test needed spikes fortification (for example, during the recovery study), they were done in this stage. The QuEChERS method was performed according to Anastassiades [14] (the solvent and quantity of reagents were optimized). 8 mL of n-hexane was added and the tube was shaken for 3 min (Agitation 01). Then added 0.5 g of sodium chloride and 1 g of magnesium sulphate and shaken for 3 min (Agitation 02), phase separation was achieved. After it was centrifuged at 3500 rpm for 10 min at a temperature of 5°C an aliquot of 3 mL of the n-hexane phase was transferred to a 15 mL Falcon tube with 0.5 g of magnesium sulphate 0.05 g of PSA and 0.075 g of C_{18} and shook for 1 min (Agitation 03). Then it was centrifuged at 3500 rpm for 10 min at a temperature of 5°C. After that 1 mL of supernatant was transferred to a vial prior to analysis.

Instrumentation

GC/ECD: OCs were analysed using a Trace GC Ultra with an ECD apparatus (Thermo Fisher Scientific), equipped with a capillary column of 30 m, OV-5MS (0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed starting at 80°C and held for 15 min followed by increases of 40°C min^{-1} to 170°C, then 65°C min^{-1} to 220°C and held for 7 min, then 15°C min^{-1} to 265°C held for 4 min, then 25°C min^{-1} to 310°C held for 8 min. The injection port was at 250°C in splitless mode, and the detection was carried out at 300°C. Helium was used as carrier gas at a constant flow rate of 0.8 ml min^{-1}; while nitrogen (purity ≥ 99.999%) was employed as a make-up gas at the flow of 30 ml min^{-1}. An automatic injector AS 3000 was employed at an injection volume of 1 µL.

GC/MS: a single quadruple gas chromatograph-mass spectrometer GC/MS QP 2010 (Shimadzu Corporation) operated in electron impact ionisation (EI) mode at 70 eV was used for confirmation of the OCs and it was equipped with a column OPTIMA-5 (30 m × 0.25 mm, 0.25 µm film thickness). The injector was operating in splitless mode and an ultrapure grade helium (purity ≥ 99.999%) were used as the carrier gas at 1.0 mL min−1 flow. The GC oven temperature was programmed from an initial temperature of 80°C (15 min hold), ramped at 40°C min^{-1} to 170°C, then 65°C min^{-1} to 220°C (15 min hold) and finally at 8°C min^{-1} to 265°C with holding for 4 min. The other optimised parameters included a transfer line temperature of 250°C and an ion source of 220°C. An automatic injector AS 3000 was employed, injecting 1 µL. Table 1 shows the confirmation ions of OCs.

Compound	Time (min)	Acquisition interval (min)	m/z (% rel. intensity)	m/z (% rel. intensity)	m/z (% rel. intensity)
Hexachlorobenzene (HCB)	8.68	8.55 – 8.80	284 (100)	286 (82)	249(26)
Lindanec (LIN)	9.14	8.88 – 10.00	217 (74)	183 (100)	219 (95)
Aldrinc (ALD)	11.69	11.50 – 12.00	66 (100)	220 (13)	263 (93)
Heptachlor Epoxide (HPX)	12.75	12.00 – 13.00	81 (43)	353 (100)	355 (78)
t-Chlordane (tCLD)	13.50	13.00 – 13.60	272 (19)	373 (100)	375 (99)
PCB 101	13.73	13.60 – 13.90	254 (49)	324 (59)	326 (100)
c-Chlordane (cCLD)	14.04	13.90 – 14.50	272 (20)	373 (100)	375 (99)
pp'-DDE (ppE)	14.82	14.50 – 14.88	246 (100)	316 (68)	318 (85)
Dieldrin (DLD)	14.93	14.88 – 15.50	79 (100)	263 (42)	279 (26)
PCB 118	16.36	16.00 – 16.50	254(33)	324 (64)	326 (100)
pp'-DDD (ppD)	16.72	16.50 – 16.85	165 (27)	235 (100)	237 (64)
op'-DDT (opT)	16.94	16.85 – 17.20	165(24)	235 (100)	237 (63)
PCB 153	17.56	17.20 – 18.00	290 (51)	358 (52)	360 (100)
pp' DDT (ppT)	18.94	18.00 – 19.10	165(26)	235 (100)	237 (67)
PCB 138	19.23	19.10 – 22.00	290 (52)	358 (51)	360 (100)
PCB 180	24.89	24.00 – 26.00	324 (54)	392 (42)	394 (100)
Mirex (MRX)	27.10	26.00 – 36.00	237 (45)	272 (100)	274 (77)

Table 1: Confirmation ions used in GC/MS analysis.

Data processing

The software Statistica 7 was used for calculations of design of experiments. Further calculations were made using Microsoft Excel 2013.

Results and Discussion

Optimisation

In preliminary studies, the recovery amount (70–120%) required by the standard SANCO 12571 [15-20] was not obtained and as a result, the process to optimize the extraction had to be planned. Firstly, some parameters that have low influence on the result, like volume of hexane, the mass of the matrix and the amount of magnesium sulphate were planned by univariate design. The choice of variables that have high influence on the results for the experimental design was:

Factor 01: Mass of NaCl: low level (-1) of 0.5 g and high (+1) 1.5 g, to observe the influence of ionic strength on the extraction of analytes of interest.

Factor 02: Mass of PSA: low level (-1) 50 mg and high (+1) 150 mg.

Factor 03: Mass of C18: low level (-1) 25 mg and high (+1) 75 mg in the clean-up phase method using DSPE (Dispersive Solid Phase Extraction).

From this data, a full factorial design 23 was performed using a triplicate of the central point using Statistica Software 7.0 to promote randomization and for processing the results (Table 2).

Experiments	NaCl	PSA	C18
(1)	-1	-1	-1
(5)	-1	-1	1
(10 C)	0	0	0
(4)	1	1	-1
(7)	-1	1	1
(8)	1	1	1
(2)	1	-1	-1
(6)	1	-1	1
(9 C)	0	0	0
(3)	-1	1	-1
(11 C)	0	0	0

Table 2: Matrix experiments of the full factorial design 23.

Whereas the objective of the planning was to evaluate the best extraction condition, the recovery parameter was used as a response of the design. The samples were spiked in the concentration of organochlorine compounds to achieve the maximum residue level (MRL) for fish, which are adopted in Brazil, based on international references, as shown in Table 3.

Organochlorine pesticides and PCB`s	Japan Other Fishes. Salmon and Trouts (mg/kg)*	USA FDA (action level) (mg/kg)**	USA Carc. CSF (mg/kg) EPA**	Brazil PNCRC (mg/kg)***
Chlordane (cCLD, tCLD)	0.05	0.3	0.35	0.3
Total DDT (ppD, ppE, ppT, opT))	3.0 (DDT)	5	0.34	5
Dieldrin (DLD)	0.1	0.3	16	0.3
Heptaclor e Heptachlor epoxide (HPX)	0.05 (Heptachlor only)	0.3 (Heptachlor epoxide only)	9.1	0.3 (Heptachlor epoxide only)
Endosulfan	0.004	-	-	-
Mirex (MRX)	-	0.1	-	0.1
PCBs	-	2	2	2
Aldrin (ALD)	0.1	-	-	0.1
Benzene hexacloride (HCB)	0.1	-	1.6	0.1
Lindane (LIN)	1	-	-	1
HCH	-	-	-	-
Endrin	0.005	-	-	-

Table 3: Maximum residue limits for organochlorine compounds in some countries. * Ministry of Health, Labour and Welfare (2006). ** United States Environmental Protection Agency (2000). ***Values adopted for a program of inspection of the food chain created by the Ministry of Agriculture, Livestock and Supply. The gray cells indicate the similarity of the boundaries between countries.

Fourteen analytes were analysed and their recoveries (%) obtained are presented in Table 4.

Experiments	HCB	LIN	ALD	HPX	tCLD	cCLD	ppE	DLD	PCB118	ppD	opT	ppT	PCB180	MRX
-1	83	79	82	81	80	83	93	72	87	83	92	91	86	89
-5	90	82	89	83	84	88	97	71	93	84	96	94	94	94
(10C)	87	73	87	77	75	80	94	66	89	76	91	88	89	91
-4	89	68	87	76	71	78	93	66	89	72	91	86	90	91
-7	86	64	83	70	66	74	91	57	85	66	86	82	86	90
-8	86	66	83	71	67	74	90	58	85	68	88	83	86	90

-2	81	77	80	76	76	80	90	66	84	79	88	87	84	85
-6	89	78	88	79	80	85	94	66	91	81	93	91	92	94
(9C)	92	74	91	79	76	82	94	67	90	76	91	88	91	94
-3	91	70	90	78	72	79	94	66	88	72	90	86	89	91
(11C)	92	75	90	79	76	82	95	67	88	76	91	89	92	94

Table 4: Recovery (%) results for each experiment of the design.

Based on the results, it was observed that the experiments (1) and (5) obtained recovery given the criterion for acceptance of SANCO 12571 [15] (70-120%) for all compounds, and the best average recoveries (considering all analytes) was in experiment (5). Figure 1 shows a bar graph of the effects of each parameter studied, indicating that the most significant are related to PSA and C18. Based on the results, we adopted the experiment (5) as the best condition to promote the validation of the method. It is worth noting that after planning it was also decided to incorporate the analyte-PCBs 101, 138 and 153 in this test method.

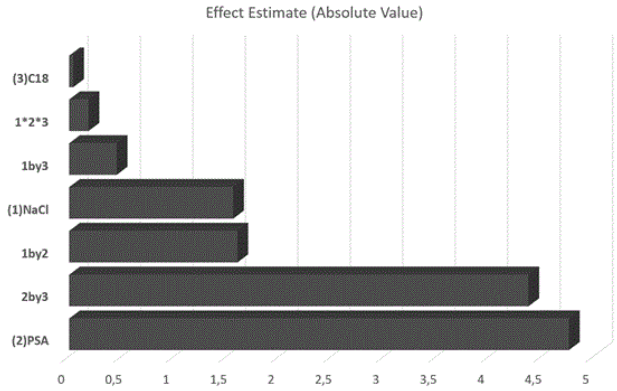

Figure 1: Bar graph of the effects.

Validation

Selectivity: Selectivity occurred throughout the validation process for the analysis of different samples of fish. In this way, six different samples were analyzed and evaluated, there was not peak interference in the retention times of the analytes that were ≥ 30% of the signal at the lowest concentration of the calibrated level. Figure 2 is an overlap of the lowest point calibrated with a sample free of analyte. The results showed that the method is selective for the analyte of interest.

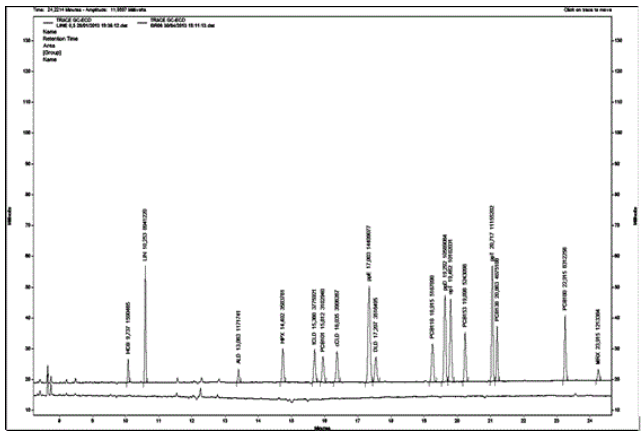

Figure 2: Overlay of two chromatograms: lower calibration point (upper base line) and a sample free of analyte (lower baseline), showing that there is no overlapping peaks. It can be noted that signal / noise ratio is much higher than 6.

Linearity: According to Konieczka and Namiesnik linearity is the ability of an analytical method to produce results, which are directly proportional to analyte concentration for samples in a given concentration range. So the working range comprises of concentrations where linearity is achieved by defining the limits of the calibration curve. The matrix extracts were spiked in six concentration levels corresponding with the MRLs for each analyte (0.5, 0.8, 1.0, 1.2, 1.5 and 2.0 times the MRL), with six replicates being analysed [18].

According to SANCO 12571 [15], acceptable criteria include the evaluation of the coefficient of determination to be higher than 0.980 (R2 > 0.980) and examination of individual residues should not be more than ± 20% compared to the response obtained in the calibration curve. When these points approach or exceed the MRL (maximum residue level), this value should not exceed ± 10%. Moreover, all analytes obtained R2 values higher than 0.980 and the highest individual residues found fluctuated between 5 and 13%, with the value of 13% belonging to a point that does not approach or exceed the MRL.

Recovery: Recovery is defined as: "the ratio of the amount of analyte present or added in the analytic portion of the matrix, which is extracted and that could be measured". Recovery was evaluated by two fortification levels (at 0.5 times MRL and 1.0 times MRL for each target analyte) in five replicates for each level. The results were compared with the acceptance criteria of SANCO 12571 [15], which requires recoveries of 70–120% with an accuracy of ≤ 20% of the coefficient of

variation (CV). All recovery results were approved, ranging from 93% to 118%.

Repeatability: Repeatability, defined as "the degree of agreement between the results obtained under the same operating conditions (same analyst, same equipment, etc.) in a short time (the same day or in two days different and next)". Repeatability was evaluated in two fortification levels (at concentration of 0.5 and 1.0 times the MRL for each analyte) and was analyzed in ten replicates for each level. The results were compared with the acceptance criteria defined in SANCO 12571 [15], which requires for precision that CV ≤ 20%. All analytes showed satisfactory results, with values between 4% and 17%.

Intermediate Precision: Intermediate Precision, defined as "condition of measurement in a set of conditions that includes the same measurement procedure, same location, and replicate measurements on the same or similar objects over an extended period of time, but may include other conditions involving changes", was evaluated in two fortification levels (the concentration 0.5 and 1.0 times the MRL) and was analyzed in ten replicates for each level, performed by two different analyst. The results were compared with the acceptance criteria defined in SANCO 12571 [15], which requires for precision CV ≤ 20%. Results fell between 2% and 13% and were all approved.

Limit of Quantification: According to SANCO 12571 [15], the limit of quantification of the assay is defined as the lowest level of fortification validated that meets the recovery criteria: 70–120% with CV ≤ 20%. For the quantification limit, the signal / noise ratio should exceed six (S/N ≥ 6).

Considering the lowest level of fortification was valid, 0.5 x MRL was considered to be the limit of quantification for the method. It was possible to obtain adequate linearity, recovery, repeatability and intermediate precision with this LoQ. The chromatogram shown above in Figure 2 was obtained at this concentration level indicating proper resolution of the chromatographic peaks value of the signal / noise above 6 for all compounds.

Confirmation in GC/MS: According to SANCO 12571 [15], the use of highly specific detection systems, such as Mass Spectrometry (MS) are recommended. MS was used to evaluate at concentrations in the MRL for analytes of interest in fish array to properly identify them. Furthermore, a study was performed where three samples were evaluated with regard to percentage change of the relative intensity between the replicates. The evaluation was done according to the criteria of SANCO 12571 [15] that it is indicated on Table 5.

Relative intensity (percentage of base peak)	Variation of Replicate
>50%	± 10%
>20% a 50%	± 15%
>10% a 20%	± 20%
≤ 10%	± 50%

Table 5: Percentage change acceptance criteria regarding the relative intensity between the replicates analysed.

For the MS analysis, three ions were selected, and at least two had m/z>200. Based on the considerations, satisfactory results were obtained and all analytes could be confirmed successfully.

Uncertainty estimation

Based on the validation results, it was possible to estimate the uncertainty in the lower calibration point. The results of repeatability, intermediate precision, recovery and calibration curve, as shown in Ishikawa diagram (Figure 3) were used. The calculations were performed following the Eurachen/CITAC Guide [19]. The results of this estimation are shown in Table 6 together with a summary of the results of the main parameters of the validation.

Analite	**Uncertainty estimation at minor level of calibration (%)**	**Selectivity** acceptance criteria: interferences ≤ 30% of LoQ	**Linearity** acceptance criteria: $r^2 \geq 0.980$	**Recovery** acceptance criteria: between 70 and 120%, with CV ≤ 20%		**Repeatability** acceptance: criteria: CV ≤20%		**Intermediate Precision** acceptance criteria: CV ≤ 20%		**Limit of Quantification (LoQ)** acceptance criteria: recovery between 70 and 120%, with CV ≤ 20%		**GC/MS Confirmation**
	-	%	r^2	0.5 LMR	1.0 LMR	0.5 LMR	1.0 LMR	0.5 LMR	1.0 LMR		0,5 LMR (mg Kg^{-1})	-
HCB	29.85	<30%	0.9996	112 (cv:13)	114 (cv:6)	10	11	6	4	50		Yes
LIN	19.98	<30%	0.9988	93 (cv:7)	95 (cv:3)	6	3	5	3	500		Yes
ALD	24.52	<30%	0.9997	118 (cv:12)	113 (cv:9)	10	9	5	2	50		Yes
HPX	24.74	<30%	0.9987	109 (cv:11)	108 (cv:8)	10	7	4	5	150		Yes
tCLD	21.66	<30%	0.9987	102 (cv:10)	107 (cv:6)	8	6	4	6	150		Yes
PCB101	28.23	<30%	0.9996	118 (cv:13)	116 (cv:7)	11	8	5	5	200		Yes
cCLD	25.16	<30%	0.9988	108 (cv:12)	109 (cv:6)	10	6	3	6	150		Yes

ppE	19.06	<30%	0.9987	113 (cv:7)	109 (cv:3)	6	4	3	6	625		Yes
DLD	21.85	<30%	0.9994	107 (cv:10)	105 (cv:5)	8	6	5	6	150		Yes
PCB118	22.13	<30%	0.9983	115 (cv:10)	110 (cv:5)	7	6	3	6	200		Yes
ppD	22.95	<30%	0.9967	97 (cv:7)	95 (cv:4)	7	7	4	5	625		Yes
opT	20.09	<30%	0.9972	111 (cv:7)	106 (cv:3)	5	5	3	5	625		Yes
PCB153	21.65	<30%	0.9979	115 (cv:8)	109 (cv:5)	7	5	5	5	200		Yes
ppT	23.76	<30%	0.9973	105 (cv:6)	102 (cv:3)	8	6	6	4	625		Yes
PCB138	24.59	<30%	0.9974	113 (cv:9)	108 (cv:5)	8	5	6	5	200		Yes
PCB180	38.13	<30%	0.9986	110 (cv:9)	108 (cv:4)	17	6	13	6	200		Yes
MRX	38.09	<30%	0.9999	109 (cv:18)	107 (cv:9)	16	8	9	7	50		Yes

Table 6: Summary of validation results.

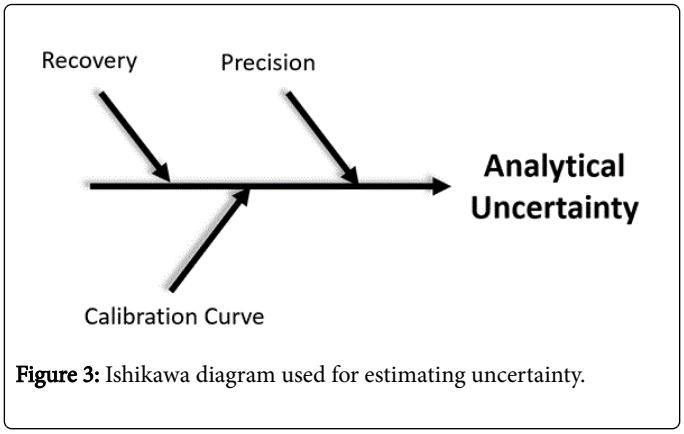

Figure 3: Ishikawa diagram used for estimating uncertainty.

Certified Reference Material

The certified reference material NIST 1946 was analysed by the validated method after a confirmation of two parameters evaluated: linearity (required for quantification) and recovery (used as a correction factor for the results). The results of linearity and recovery are presented in Table 7.

	Linearity		Recovery 70–120%
Analites	**r^2>0.980**	**Major Residue< ± 20%**	
HCB	0.998	-5	93.83
LIN	0.982	-12	84.3
ALD	0.996	-9	96.42
HPX	0.992	-10	92.53
tCLD	0.992	-10	92.56
PCB101	0.994	-8	96.7
cCLD	0.996	-7	96.08
ppE	0.991	-7	99.11
DLD	0.996	-8	92.84
PCB118	0.998	-3	98.94
ppD	0.998	2	89.29
opT	0.998	3	97.69
PCB153	0.998	-3	98.81
ppT	0.998	2	95.22
PCB138	0.997	2	96.61
PCB180	0.991	6	94.84
MRX	0.989	-6	94.85

Table 7: Results for linearity and recovery, when analyzing the MRC NIST 1946.

The certified concentrations for the analytes present in the CRM were below the lowest calibrated level (0.5 times the MRL) and it is not possible to quantify by the calibration curve. However, the recommendation of SANCO 12571 [15] was considered, where a single-level calibration may be used provided that the sample signal be ± 50% of the signal for the calibrated level. Based on this guidance, it was possible to quantify analytes: p, p-DDE, PCB 153 and PCB 138, which had respective signals of 59.68%; 85.00% and 57.50% of the signal to the lowest calibrated level.

The uncertainty of the results regarding the analysis of the CRM (uncertainty of the laboratory) were calculated using the "validation-based" approach, so the results of repeatability, intermediate precision and recovery (the uncertainty of the calibration curve was not used, since the results were calculated using single-level calibration) were used.

To evaluate the test results, the reference material was used to calculate the normalized error as shown in Equation 1 and Table 8, it is considered satisfactory when less than 1, according to ISO 13528 [20].

Analite	**CRM ($\mu g.kg^{-1}$)**	**CRM uncertainty ($\mu g.kg^{-1}$)**	**Obtained Value ($\mu g.kg^{-1}$)**	**Uncertainty of the Obtained**	**Normalized Value**

				Value ($\mu g.kg^{-1}$)	
ppE	373	± 48	374	± 52	0.01
PCB153	170	± 9	153	± 22	-0.71
PCB138	115	± 13	129	± 23	0.53

Table 8: Results for Certified Reference Material analyzed.

Conclusion

Considering how demand for quality assurance (QA) has grown in analytical laboratories [21], it's possible to note that there are different ways to apply statistical tools (like design of experiments and validation), and the incorrect use of these tools can affect the quality of the results that will be produced by the method validated. This way, the QuEchERS method could be developed and validated by using appropriated statistical tools, like design of experiments and validation parameters (repeatability, selectivity, intermediate precision, linearity, among others). The use of certified reference materials to confirm the accuracy and precision of the result is very important but it needs to be associated to the uncertainty evaluation of the result generated to achieve a comparison amongst the certified value and the result of the method, reaching metrological traceability. Finally, the development of the method in a laboratory that operates according ISO/IEC 17025:2005 contributes not only with the metrological but documental traceability too.

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