Determining organochlorine pesticides in samples of green sea turtles by QuEChERS approach

Determinação de pesticidas organoclorados em amostras de tartarugas verdes pelo método QuEChERS

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Abstract

Some Organochlorine Pesticides (OCPs) can pose numerous adverse effects on biota. Marine turtles face numerous threats, in particular those related to anthropogenic activities. Therefore, development and improvement methodologies for monitoring chemical compounds are a relevant task. In this work, we developed a methodology based on the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction for detection of twelve OCPs, by gas chromatography with electron capture detector, in fat and liver samples of green sea turtles. Quantification limits were lower than 5.3 ng g^{-1}; acceptable recovery rates for most compounds; medium matrix effect; matrix-calibration with linearity at the range from 1.0 to 200 ng g^{-1}. This methodology provides contributions for the study of pesticide residues with adverse effects on sea turtle health, important skills for new directions in conservation issues.

Keywords: *Chelonia mydas*. Electron capture detector. Fat. Liver. Persistent Organic Pollutants (POPs).

Resumo

Alguns Pesticidas organoclorados (OCPs) podem causar numerosos efeitos adversos na biota. As tartarugas marinhas enfrentam diversas ameaças, em especial aquelas relacionadas às atividades antropogênicas, por isso o desenvolvimento de melhorias nos métodos para monitorar compostos químicos são tarefas importantes. Neste trabalho foi desenvolvida uma metodologia baseada na extração QuEChERS (*Quick, Easy, Cheap, Effective, Rugged and Safe*) para a detecção de doze OCPs, por cromatografia gasosa com captura de elétrons, em amostras de gordura e fígado de tartarugas verdes. Os limites de quantificação ficaram abaixo de 5.3 ng g^{-1}; com taxas de recuperação aceitáveis para a maioria de compostos; efeito matriz médio; calibração da matriz com linearidade variando de 1.0 a 200 ng g^{-1}. Esta metodologia traz contribuições ao estudo de resíduos com efeito adverso na saúde das tartarugas marinhas, sendo importante instrumento para novas direções em temas de conservação.

Palavras-chave: *Chelonia mydas*. Detector de captura de elétrons. Gordura. Fígado. Poluentes Orgânicos Persistentes (POPs).

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Introduction

Tissue residue levels can be considered as biomarkers, endpoints that can be used to evaluate exposure or effects of chemical stressors (VENTURINO et al., 2003). Several Organochlorine Pesticides (OCPs) are considered Persistent Organic Pollutants (POPs) and are found in all compartments of ecosystem, including the long-lived chelonians, especially in tissues with high fat contents, highlighting the big risk because of the wide range of acute and chronic effects on those organisms (AGUIRRE; LUTZ, 2004; KELLER et al., 2004). It has been well established that marine turtles are affected by anthropogenic activities and pollution, involving diseases such as the fibropapillomatosis (FP). The monitoring of OCPs may help to understand its role in development of diseases providing a basis for research, prediction and mitigation of these episodes (STORELLI; MARCOTRIGIANO, 2000). Moreover, it has a potential conservation and human health implications by predicting the effects of these chemicals on sea turtle populations and by rising human health considerations in areas with consumption of products derived from those species (VAN DE MERWE et al., 2009).

With respect to analytical questions, extraction of organic pollutants can be laborious in complex matrices such animal tissues and the analytical methodologies continuously require adaptations. Thus, the improvement of methodologies remains a necessary task. Simple and rapid methods stand out in this context, because they are less dependent on high investment. In this way, the current study aimed to adjust a protocol based on the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction approach (ANASTASSIADES et al., 2003) as performed by Castillo et al. (2011), for determining twelve OCPs in fat and liver samples of green sea turtles *(Chelonia mydas)*.

Materials and Methods

Specimens of *C. mydas* died as bycatch in October 2011 were captured at Praia Grande-SP, Brazil by Projeto Biopesca. Fragments of 5-10 g of fat and liver were collected and wrapped in individual aluminum foils and stored at -20°C. These samples were used as blank. All procedures were developed in accordance with the *Comissão de ética no uso de animais* – Universidade de São Paulo (CEUA: 2116/2010) and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Ministério do Meio Ambiente (MMA) (SISBIO 26667-1).

Twelve pesticides (α-BHC, β-BHC, Heptachlor, α-Endosulfan, β-Endosulfan, Endosulfan sulfate, PP' -DDD, OP' -DDD, PP' -DDE, OP' -DDE, Dicofol and Mirex) were selected because of historical use and economical interest in Brazil. Materials were purchased as follows: analytical standards with purity > 98,5%: (Dr. Ehrnstorfer®, Augsburg, Germany and ChemService®, West Chester, PA, USA); solvents having purity > 98 %, HPLC degree (J.T. Baker, Tedia Company Inc.® and Macron TM Chemicals®); magnesium sulfate anhydrous - $MgSO_4$, Reagent plus, ≥99,5% (Sigma-Aldrich®), Primary Secondary Amine (PSA, Agilent Technologies®), silica gel (ø mm 0,05-0,20 RS, apparent specific density (g L^{-1}) 400÷440 (Analyticals®, Carloerba), sodium sulfate anhydrous - Na_2SO_4 (Mallinckrodt AR®) and purified water at Milli-Q Academic system from Millipore®.

A chromatographic gas phase system (GC) (Agilent 7890A) equipped with autosampler (Agilent 7683), capillary column Agilent HP-5 (5% Fenil Metil Siloxane) (30 m x 320 μm x 0.25 μm), micro electron capture detector (μECD) and ChemStation B.04.02 software was used. Injector in *pulsed-splitless* mode at 280°C; oven initial temperature was set at 100°C, up to 210°C (at 20°C min^{-1} held for 3 min); 210°C, up to 230°C (at 15°C min^{-1} held for 5 min), 230°C, up to 280°C (at 10°C min^{-1} held for 3 min); carrier gas N_2 with constant flow of 1 mL min^{-1}; temperature detector of 300°C; *make-up* gas N_2 of 39 mL min^{-1}. Total analytical run time was 22.8 min.

Adjusting the protocol of Castillo et al. (2011) based on d-SPE (*dispersive solid-phase extraction*), the analytes were first extracted from 1g (Ultraturrax homogenized sample) using acetonitrile saturated with n-hexane (18%), followed by a pre clean-up by freezing the liquid phase (in order to induce fat precipitation), a first clean-up based on QuEChERS and a second clean-up using a mini column of silica gel (instead of C18 in the SPE dispersive purification) (GEBARA et al., 2005) (Figure 1).

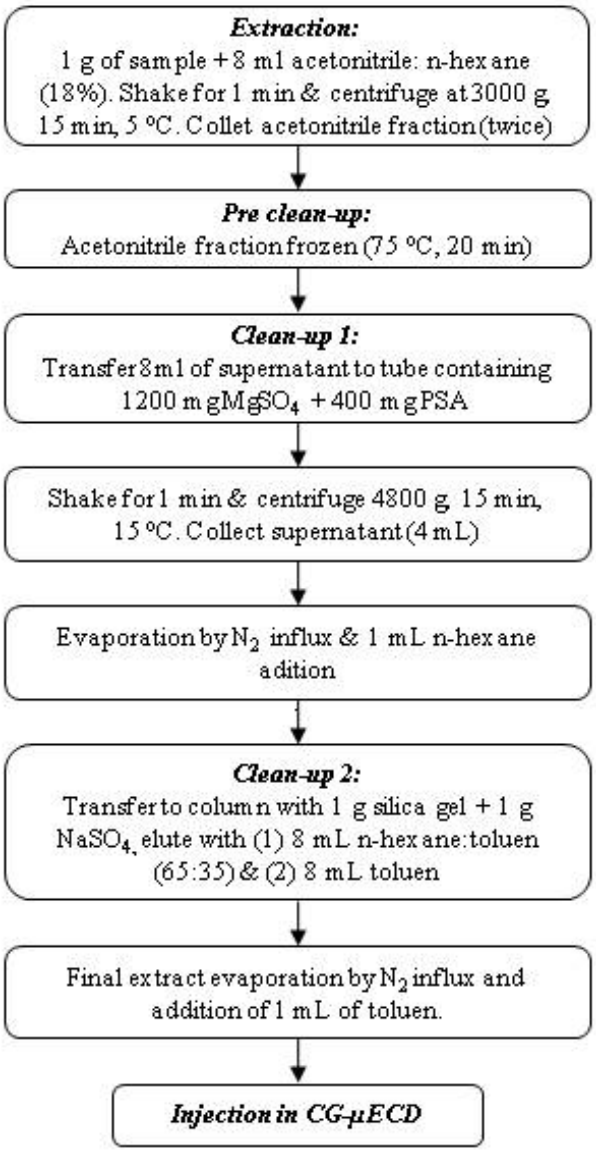

Figure 1 – Flowchart of the analytical experiment

The validation of the proposed procedure was carried out considering the following parameters: matrix effect, specificity, limits of detection (LODs) and quantification (LOQs), precision and accuracy. Matrix matched calibration were set as seven points: 1; 2; 10; 20; 40; 100 and 200 ng g^{-1}. The angular and linear coefficients and determination coefficients were calculated by applying the Huber test (k = 2). The specificity was shown in the blank and fortified samples. LODs and LOQs were calculated as three and 10 times, respectively, the signal-to-noise ratio (S/N) in relation of lowest point of calibration curve. Peaks were quantified as target compounds when matched the retention time within ± 5% of the standard compound (MALARVANNAN et al., 2011). The recovery study was done by seven replicates of both matrices fortified at 6 ng g^{-1} and 60 ng g^{-1}. Intermediate accuracy was evaluated from recovery tests from seven replicates with fortified samples at 60 ng g^{-1} concentration with an interval of seven days, through RSD% calculation between dates. Student's *t*-test was applied to verify the existence of significant differences between averages at 99% (t=3.14). Comparison between slope ratios of standards in solvent and matrix were performed to determine matrix effect (ME) (MAGNUSSON; ÖRNEMARK, 2014).

Results and Discussion

All LODs and LOQs values were below 3.1 and 5.3 ng g^{-1} wet weight (w/w) respectively. The working range of choice presented excellent linear relationship with the analytical signal, as indicated by the determination coefficient (r^2) higher than 0.99 for all compounds. Comparison between slope ratios of standards in solvent and matrix were performed to determine matrix effect (ME) (MAGNUSSON; ÖRNEMARK, 2014): medium matrix effect was recorded with signal suppression in both matrixes (values between 20% and 40%) and proved that clean-ups were satisfactory, considering the sample complexity. Nevertheless, the quantification of

compounds was developed employing the line equations in analytical matrix matched calibration.

Recovery values and other validation parameters were acceptable for most compounds (Table 1). The use of GC-μECD for determination and quantification of OCPs is known to be highly sensitive, as observed in the present study. Nowadays, the GC-MS/MS technique is highly sensitive like the GC-μECD but is more expensive and requires less accessible equipment.

The results obtained in this investigation for LODs, LOQs and recoveries of OCPs in tissues of sea turtles were, in general, comparable to those already reported. Noteworthy, comparisons between studies must be done with caution, due to the existence of several factors relative to analytical procedures involved in results (such as equipment, reagents, analytes analyzed and matrix; including sea turtle species). Gardner et al. (2003) defined LODs as 3.0 ng g^{-1} w/w for 21 OCPs (6 DDT isomers, seven Chlordanes, three Cyclodienes, Hexachlorobenzene (HCB), Lindane and α and β Endosulfan) in liver and adipose tissues from three species of sea turtles. For recovery on the matrix, 80% of the samples had to have values between 50% and 150% of the assigned value and, in duplicate, this relative percent was 50% for 80% of the analytes. Keller et al. (2004) set LODs as 1 ng g^{-1} w/w for determination of three Chlordanes, three DDT isomers, Dieldrin and Mirex in adipose tissue in fat and liver of loggerhead sea turtles (*Caretta caretta*). In the same species, Rybitski et al. (1995) reported LOQs were approximately 2 μg kg^{-1} and mean recoveries of Decachlorobiphenil (DCB) as 95.4% (SD = 27.3%) in subcutaneous fat and 82.6% (SD = 26.7%) in liver. Lazar et al. (2011) determined PCBs and some OCPs (HCB, α-HCH and 3 DDT isomers) in yellow fat and reported overall recoveries ranged from 51% to 68% with relative standard deviation from 18 to 29% and depending on the compound LODs ranged from 0.1 to 0.3 μg kg^{-1} lipid weight (l/w). Malarvannan et al. (2011) stabilized lower LODs for OCs and polybrominated diphenyl ethers (PBDEs) in liver of some sea turtle species at the range of 0.05-1.5 and 0.01-0.02 ng g^{-1} l/w and the recovery for DDTs, PCBs, CHLs, HCHs were 102 ± 4.1%, 101 ± 4.3%, 103 ± 1.5% and 99 ± 1.9%, respectively. In Storelli and Marcotrigiano (2000), LOQs results ranged from 0.1 to 0.4 ng g^{-1} w/w and recoveries were within 80-110% for 5 DDT isomers, HCB and PCBs in various tissues of *C. caretta*.

In conclusion, this study provides contributions on analytical methodologies for determining residues in samples of green sea turtles using a method based on QuEChERS and GC-μECD. The proposed methodology allowed the determination and quantification of OCPs in the adipose and hepatic tissue of *C. mydas* at specific conditions. Further analysis of sea turtle tissues aiming to add knowledge about the status of chemical pollutants and assess its effects on the health of these animals is vital to provide skills for new direction in conservation.

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Table 1 – Results of pesticides validation parameters in "fat" and "liver" of green sea turtles (*Chelonia mydas*) – São Paulo – February 2015

Matrix	Compound/ Validation parameter		α-BHC	β-BHC	Heptachlor	Dicofol	op'-DDE	α-Endosulfan	pp'-DDE	op'-DDD	β-Endosulfan	pp'-DDD*	Endosulfan sulfate	Mirex
	Retention time (min)		7.80	8.30	9.90	10.7	12.3	12.6	13.3	13.6	14.7	14.9	16.3	19.4
Fat	**Matrix effect (%)**		26.9	31.7	32.8	42.1	34.4	32.8	32.0	31.2	31.1	31.5	31.6	39.0
	LOD ng g^{-1}		2.00	0.80	3.10	1.30	0.4	1.50	1.90	0.40	0.50	0.50	0.44	1.40
	LOQ ng g^{-1}		2.10	1.20	5.30	1.60	0.6	1.80	2.00	0.90	0.60	0.60	0.60	2.40
	Recovery % (n=7) / RSD %	**6 (ng g^{-1})**	171	174	148	27.7	88.0	124	131	117	129	153	81.6	103
			± 14.1	± 25.4	± 26.0	± 20.1	± 8.5	± 11.9	± 23.5	± 23.4	± 6.20	± 21.7	± 21.5	± 11.2
		60 (ng g^{-1})	98.5	158	65.5	130	101	110	88.0	121	120	110	121	58.4
			± 8.90	± 7.40	± 6.60	± 8.10	± 10.4	± 6.70	± 8.40	± 5.90	± 3.70	± 5.90	± 5.0	± 17.2
	Intermediate precision (n=7)/ RSD % / 7 days	**60 (ng g^{-1})**	103	166	68.2	115	102	112	91.3	123	125	110	124	90.7
			± 9.00	± 4.90	± 18.1	± 10.9	± 9.5	± 5.70	± 8.80	± 6.30	± 4.10	± 6.10	± 5.3	± 7.40
Liver	**Matrix effect (%)**		26.8	30.9	40.5	44.7	30.3	31.1	29.8	29.6	29.3	30.9	26.4	39.3
	Recovery % (n=7) / RSD %	**6 (ng g^{-1})**	181	80.1	147	123	152	146	132	138	145	158	186	47.7
			±15.2	± 29.0	± 31.2	± 18.0	± 38.6	± 14.4	± 5.8	± 9.90	± 4.80	± 5.00	± 16.0	± 15.2
		60 (ng g^{-1})	74.9	134	56.3	177	108	109	93.5	116	127	103	125	76.7
			± 27.5	± 13.8	± 36.5	± 29.1	± 13.0	± 8.70	± 10.5	± 16.1	± 6.50	± 18.7	± 8.30	± 17.9
	Intermediate precision (n=7)/ RSD % / 7days	**60 (ng g^{-1})**	77.3	133	60.9	138	111	114	96.4	112	134	101	117	89.1
			± 27.6	± 18.5	± 35.5	± 33.4	± 14.4	± 10.1	± 11.3	± 18.2	± 8.90	± 19.7	± 12.1	± 21.8

LOD = limit of detection, LOQ = limit of quantification; n = number of replicates; RSD = Relative Standard Deviation. ng g^{-1} wet weigth (w/w).* limits were equal for all compounds but pp'-DDD in liver: LOD = 2.80 and LOQ = 2.90 respectively

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