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Dedicated to Professor Emil Cordoș on the occasion of his 80th anniversary

DETERMINATION OF THE ORGANOCHLORINE PESTICIDE RESIDUES CONTENTS IN GRAPES BY SBSE-TD-GC-ECD ANALYSIS

IOAN SIMON[a], MIRELA MICLEAN[b*], OANA CADAR[b], LĂCRIMIOARA SENILA[b]

ABSTRACT. Stir bar sorptive extraction (SBSE)-thermal desorption (TD) procedure combined with gas chromatography electron capture detection (GC–ECD) was applied to the determination of 20 organochlorine pesticides (OCPs) in six white Romanian grape varieties. Analyses were performed using stir bars coated with 1.0 mm polydimethylsiloxane. The method provided satisfactory analytical performance to monitor OCPs in grape matrices at the trace level. By using the standard addition methodology, good linearity (r^2>0.99) was found for all cases, depending on the particular OCP and also good sensitivity was achieved for all the investigated OCPs in agreements with the European Union regulations for the maximum residue limits (MRLs) of pesticides in agricultural vegetables. The method has multiple advantages, such as: simplicity, almost solventless and requires low sample amount, in comparison with conventional methods of sample preparation to analyse pesticides in vegetable matrices. The obtained results showed that OCPs were detected in all the investigated grape samples, with total contents varied between 0.32 µg/kg and 3.48 µg/kg, the concentrations were much lower than their specific MRLs.

Keywords: *organochlorine pesticides, stir bar sorptive extraction, GC-ECD, grapes*

INTRODUCTION

Pesticides are widely used in fruit and vegetables growing, because of their susceptibility to insect and diseases attacks. Most of organochlorine pesticides have been banned in many countries because their toxicity to

[a] *University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca, Department of Surgery, 18 Republicii Street, 400015 Cluj-Napoca, Romania, isimon@umfcluj.ro*
[b] *INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath, 400293 Cluj-Napoca, Romania.*
[*] *Corresponding author: mirela.miclean@icia.ro*

humans, but because of their considerable stability in the environment (as long as 30 years), their residues still appear as contaminants in food, as well as in the environment [1, 2].

Organochlorine pesticides (OCPs) are the most persistent organic contaminants in the environment, being classified as persistent organic pollutants (POPs) due to their ubiquity, persistence and bioaccumulation in the environment [3]. The high toxicity of OCPs poses significant threats to human health and biodiversity [4]. Recent reports identified an association between exposure to pesticides and different types of human cancer [5]. Many of the OCPs are known as endocrine disruptors, also they cause immune suppression and inhibit various enzymes. DDT has been reported to affect neurobehavioral functions and to be associated with premature births [6].

Agricultural soils are important reservoirs for OCPs due to their tremendous retention capabilities for these compounds and they can enter the food chain directly through absorption into vegetation [4]. Also, OCPs can be transferred from air and atmospheric particulates into the vegetables [7]. Therefore, residues of pesticide could affect the consumers especially when these commodities are freshly consumed [1].

Grape production is an important activity due to the high nutritional properties of grapes, being consumed both as fresh and as processed products [8]. The increase of fruit intake contributes to the prevention of chronic diseases, but could also significantly increase pesticide exposure and may thus be of health concern [9].

In order to measure the low concentrations of OCPs residues in fruit samples, highly selective, sensitive and accurate analytical methods are needed [8]. Sample preparation still remains a critical step, being complex, laborious and time-consuming, especially for the biological matrices. Initially, classical techniques of sample preparation, such as Soxhlet and (solid)liquid-liquid extraction, that employed large amounts of toxic organic solvents and generate environmentally hazardous waste were used [10, 11]. In the last decades, various microextraction methods have been innovatively employed for effective concentration of OCPs in liquid samples, before instrumental analysis. These include, among others, single drop microextraction, dispersive liquid-liquid microextraction, solid-phase microextraction (SPME), stir bar sorptive extraction (SBSE) followed by thermal desorption [12]. These methods are more environmental friendly, in agreement with modern green chemistry and analytical principles [13]. In European Union, there are several standardized methods for pesticides residues determination in foods of plant origin by GC or LC-MS/MS, after extraction with organic solvents and clean-up with different techniques [14, 15, 16].

SBSE is a solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices using a thick

film of polydimethylsiloxane (PDMS). This technique is based on the same mechanisms of SPME, but SBSE enables a much higher capacity because of the larger amount of polymeric phase compared to SPME [17, 18].

For quantitative analysis, most determinations of OCPs have been developed using chromatographic techniques due to their high resolution capacity and the availability of selective detectors, such as gas chromatography (GC) with electron capture detector (ECD) because of the halogen atoms in their chemical structure [1, 19]. Also, the use of multi-residue methods capable of analysing large numbers of pesticides in one single run is efficient approach [8].

The purpose of this study was to determine the levels of 20 OCPs (α-, β-, γ-, δ-, ε-isomers of hexachlorocyclohexane, 1,1,1-trichloro-2,2-bischlorophenylethane (DDT), 1,1-dichloro-2,2,-bischlorophenylethane (DDD), and dichlorodiphenylchloroethylene (DDE), each with their isomers 4,4′- and 2,4′-, also aldrin, dieldrin, heptachlor, heptachlor epoxide (isomer A), heptachlor epoxide (isomer B), alfa-endosulfan, beta-endosulfan, hexachlorobenzene (HCB) in six white grape varieties samples collected in a vineyard situated in the central part of Romania, using by SBSE, followed by thermal desorption (TD)-GC-ECD methodology.

RESULTS AND DISCUSSION

The method was validated by assessing linearity and precision. The accuracy of the method was calculated in terms of recoveries, using fresh grape samples (Sauvignon Blanc variety) fortified with standard pesticides mixture at 100 µg/kg. Limits of detection (LODs) and quantification (LOQs) were calculated as the concentration of OCPs in low level spiked matrix giving the response with a signal/noise ratio of 3 and 10, respectively.

The linearity of OCPs calibration plot was investigated over a concentration range of 0.1–100 µg/kg. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte / peak area of IS) to the relative concentration of analytes (concentration of analyte / concentration of IS). The matrix-matched standards were used for all quantification purposes to avoid any ambiguity.

The correlation coefficient (r^2) for each pesticide was greater than 0.99, indicating good linearity, as listed in Table 1.

The recovery was evaluated by spiking pesticides standards in grape sample at level of 100 µg/kg. The non-spiked and spiked samples were analyzed by SBSE, followed by TD–GC–ECD. The recoveries were calculated by subtracting the results for the non-spiked samples from those for the spiked samples. These QC samples were quantified against the matrix spiked calibration curve. The recovery rate was replicated three times and the obtained data are presented in Table 1.

Table 1. Mean recoveries (%) and relative standard deviations (RSDs) of GC–ECD determination of 20 OCPs spiked (100 µg/kg) in grape samples

OCPs	Recovery (% ± RSD)	r^2	LOD (µg/kg)	LOQ (µg/kg)
Hexachlorobenzene (HCB)	82.5 ± 4.6	0.9965	0.08	0.28
α-HCH	86.2 ± 5.1	0.9918	0.37	1.36
Pentachloronitrobenzene	88.4 ± 6.7	0.9930	0.08	0.25
γ-HCH	80.5 ± 11.0	0.9884	0.10	0.36
β-HCH	83.4 ± 4.9	0.9924	0.10	0.38
Heptachlor	73.4 ±13.0	0.9901	0.08	0.28
δ-HCH	61.1 ±20.9	0.9954	0.35	1.30
ε-HCH	76.2 ± 15.7	0.9944	0.38	1.30
Aldrin	71.8 ± 14.5	0.9953	0.04	0.15
Heptachlor epoxide β	90.5 ± 9.2	0.9941	0.03	0.10
Heptachlor epoxide α	86.2 ± 8.4	0.9912	0.05	0.18
α-Endosulfan	93.6 ± 5.0	0.9944	0.43	1.50
2,4'-DDE	51.7 ± 13.8	0.9884	0.20	0.66
4,4'-DDE	60.1 ± 18.3	0.9946	0.08	0.30
Dieldrin	98.5 ± 12.5	0.9977	0.08	0.26
2,4'-DDD	72.9 ± 19.0	0.9928	0.05	0.18
4,4'-DDD	79.0 ± 16.7	0.9945	0.08	0.28
2,4'-DDT	70.8 ± 18.4	0.9964	0.90	3.10
β-Endosulfan	89.1 ± 8.1	0.9948	0.48	1.60
4,4'- DDT	67.9 ± 24.0	0.9980	0.85	2.81

Figure 1 presents the SBSE-TD-GC-ECD chromatogram of OCPs in FA grape sample.

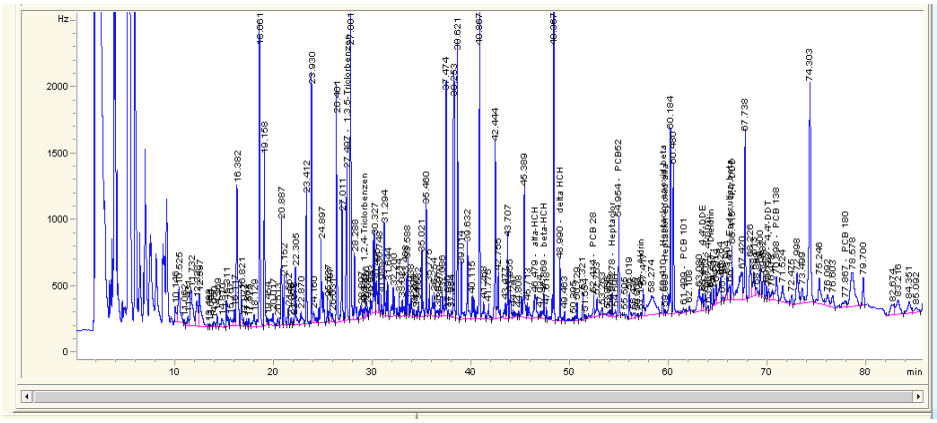

Figure 1. SBSE-TD-GC-ECD chromatogram of OCPs in FA grape sample

The limits of quantification varied between 0.10 µg/kg g (heptachlor epoxide β) and 3.10 µg/kg (2,4'-DDT) and are in compliance with the European Union regulations for the maximum residue limits (MRLs) of OCPs in agricultural vegetables [20, 21].

The recovery rate for the 20 OCPs were within acceptable range [22], with values between 60.1% (4,4'-DDE) and 98.5% (dieldrin), except for 2,4'-DDE with value of 51.7%.

The obtained results shown in Table 1 indicated that the method SBSE-TD-GC-ECD applied for grape matrix gave satisfactory performance for multiresidue analysis of 20 OCPs.

The SBSE-TD-GC-ECD method was used to determine the concentration of 20 OCPs in six white Romanian grape varieties samples and the obtained results are shown in Table 2.

Table 2. Concentrations of OCPs in different grape varieties (µg/kg)

OCPs	RI	MO	SB	FR	FA	CH
Hexachloro benzene (HCB)	1.33	1.05	<LOQ	0.61	0.42	0.60
Pentachloronitro benzene	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
α-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
γ-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
β-HCH	0.41	0.40	<LOQ	<LOQ	<LOQ	0.39
Heptachlor	0.52	<LOQ	0.32	<LOQ	<LOQ	<LOQ
δ-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
ε-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Aldrin	<LOQ	0.19	<LOQ	<LOQ	<LOQ	<LOQ
Heptachlor epoxide β	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Heptachlor epoxide α	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
α-Endosulfan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,4'-DDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4,4'-DDE	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Dieldrin	0.35	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,4'-DDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4,4'-DDD	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2,4'-DDT	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
β-Endosulfan	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
4,4'-DDT	<LOQ	<LOQ	<LOQ	2.87	<LOQ	<LOQ

Low amounts of OCPs were detected in all grape samples, the total concentrations ranged between 0.32 µg/kg (SB) and 3.48 µg/kg (FR). In the latter, the highest contribution was given by 4,4'-DDT, with 2.87 µg/kg. The

compound that occurs most often was HCB and ranged between 0.42 µg/kg (FA) and 1.33 µg/kg (RI). Among the HCH isomers, only β-HCH was recorded in 3 of the investigated samples: RI (0.41 µg/kg), MO (0.40 µg/kg) and CH (0.39 µg/kg). Heptachlor was detected in 2 samples: RI (0.52 µg/kg) and SB (0.32 µg/kg). Aldrin and dieldrin were determined in 2 samples: MO (0.19 µg/kg) and RI (0.35 µg/kg).

For some of the investigated OCPs in grapes, the European legislation [21] set the MRLs presented in Table 3. All the obtained concentrations of OCPs in the present study were much lower than their corresponding MRL.

Table 3. Maximum residue limits for OCPs in grapes, in mg/kg

OCP	**MRL (mg/kg)**
Aldrin and dieldrin (expressed as dieldrin)	0.01
DDT (sum of 4,4'-DDT, 2,4'-DDT, 4,4'-DDE, 4,4'-DDD), expressed as DDT	0.05
Endosulfan (sum of α-, β-endosulfan and endosulfan sulfate)	0.05
Heptachlor (sum of heptachlor and heptachlor epoxide), expressed as heptachlor	0.01
Hexachlorobenzene	0.01
γ-HCH	0.01
Sum of HCH isomers, except γ-HCH	0.01

CONCLUSIONS

The determination of 20 OCPs concentrations in grape samples using SBSE followed by TD-GC-ECD was investigated. The results showed that OCPs were detected in all the investigated grape samples, with total contents varied between 0.32 µg/kg (SB) and 3.48 µg/kg (FR). The method produced satisfactory results for linearity, recovery and limits of detection, situated well below the MRLs for all determined pesticides, in compliance with the EU directives [21, 22]. Also, the method has many advantages comparing to classical methods, such as a small sample amount, simplicity, low cost and almost solventless.

EXPERIMENTAL SECTION

Samples

Samples were collected in autumn 2015, in a vineyard situated in the central part of Romania and comprises of six white grape varieties: Feteasca Regala (FR), Feteasca Alba (FA), Riesling Italian (RI), Sauvignon Blanc (SB), Muscat Ottonel (MO), and Chardonnay (CH).

The sample size was at least one kg of fresh product. The portion of raw agricultural commodity prepared as the analytical sample for determination of pesticide residues was carried out according to the Codex Alimentarius, Volume 2A, Part 1-2000. A representative portion of the analytical sample was subjected to the further analysis [1].

Reagents and materials

The solvent methanol was gas chromatography grade of quality (LGC Standards, Germany).

Standard solution (Mix Standard solution for EN ISO 6468 CERTAN, NE7550) for organochlorine pesticides, polychlorinated biphenyls and chlorobenzenes was purchased from LGC Standards GmbH, Germany and contained 36 compounds at 10 µg/mL each analyte: α-, β-, γ-, δ-, ε-isomers of hexachlorocyclohexane (expressed as HCHs), 4,4′-DDE, 2,4′-DDE, 4,4′-TDE, 2,4′-TDE, 2,4′-DDT and 4,4′-DDT (expressed as DDTs), aldrin, dieldrin, heptachlor, heptachlor epoxide (isomer A), heptachlor epoxide (isomer B), α-endosulfan, β-endosulfan, hexachlorobenzene (HCB) (and also other 16 compounds which were not determined in this study. Internal standard, 1-bromo-2-nitrobenzene was also acquisitioned from LGC Standards GmbH, Germany was used in concentration of 100 µg/kg. An intermediate standard mixture of 1 µg/mL was prepared by diluting the stock standard solution, from which the working calibration standards were prepared by appropriate serial dilution with methanol. For quantification, a series of six matrix matched standards (0.1, 0.5, 1.0, 10, 20, 50, 100 µg/kg sample) were freshly prepared by the addition of the appropriate working pesticide standard solutions to the grape sample berries, before homogenization and ultrasonication, which were subjected to SBSE extractions, then to TD-GC-ECD analysis, using the procedure described in Section “Sample preparation”. Before preparation of the matrix matched standards, the grapes were analyzed in order to determine the concentrations of the targeted pesticide residues.

For SBSE extractions, SBSE stir bars (Twister, Gerstel, Müllheim an der Ruhr, Germany), 10 mm long, coated with a 1.0 mm polydimethylsiloxane (PDMS) layer were used. Prior to use, the stir bars were conditioned for 4 h at 300 °C in a flow of helium.

For the extraction, 20 mL headspace vials from Agilent Technologies (Palo Alto, CA, USA) were used.

Instrumentation and operating conditions

A thermo-desorption unit (TDU) (Gerstel) was installed on top of an Agilent Technologies 7890A gas chromatograph (GC) with electron-capture detector (µ-ECD) equipped with a programmed temperature vaporization (PTV) injector (Gerstel).

Splitless thermal desorption was performed by programming the TDU from 40 to 280 °C (5 min) at a rate of 60 °C/min. The analytes were cryo-focused in the PTV at -150 °C with liquid nitrogen prior to injection. An empty baffled liner was used in the PTV injector. For splitless injection, the PTV was ramped from -150 to 280 °C (2 min) at a rate of 600 °C /min. The used capillary column was a 30 m × 0.32 mm I.D. × 0.25 µm film thickness, coated with cross-linked 5% phenyl methyl polysiloxane was used (HP-5, Agilent J&W). The injection port temperature was 280 °C and the detector temperature was 300 °C. The column temperature program consisted of 4 stages: from 80°C to 196°C (rate 4°C/min, 2 min), from 196°C to 224°C (rate 4°C/min, 2 min), from 224°C to 240°C (rate 4°C/min, 2 min) and from 240°C to 275°C (rate 4°C/min, 2 min). High purity Helium was used as carrier gas. Blank runs of the stir bar were carried out before and after each analysis to verify the absence of any carry-over effect.

Ultrapure water (18.2 MΩ cm) was prepared by a Direct Q UV 3 Millipore system (Bedford, MA, USA).

The following instruments were used for homogenization, an Ultra-Turrax (Ika, Germany) mixer, for ultrasonication, an ultrasonic bath (Sonorex, Bandelin, Germany) and for centrifugation, a Universal 320 centrifuge (Hettich, Germany).

Sample preparation

The used method was previously described by Sandra et al. [17], with small modifications. Briefly, to the accurately weighted fruit samples (approximately 15 g), 30 mL methanol was added, then the mixture was homogenized and ultrasonicated for 15 min. A fraction (10 mL) was centrifuged (5 min at 5000 rpm) and then 1 mL of the supernatant methanol phase was extracted using a SBSE stir bar in a 20 mL headspace vial, after addition of 10 mL of ultrapure water, for 180 min with a stirring rate of 1000 rpm and at room temperature. After extraction, the stir bar was introduced in the liner of the thermal desorption system (TDU), followed by the GC-ECD analysis.

All assays, following the entire procedure, SBSE-TD-GC-ECD were performed in triplicate and blank assays were also performed using the same procedure and grape samples without spiking.

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