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Article

Development and Application of Molecularly Imprinted Polymers for the Selective Extraction of Chlordecone from Bovine Serum

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Abstract: The widespread use of chlordecone (CLD), an organochlorine pesticide, until the 1990s to protect banana crops in the French West Indies led to significant pollution of water and soil and, subsequently, of bovine intended for human consumption. Carcasses are submitted to official controls based on perirenal fat CLD determination. In order to allow for pre-slaughter controls, a selective analytical method based on a molecularly imprinted polymer (MIP) associated to the LC/MS-MS method was developed to determine the level of CLD in bovine serum that can be collected before slaughter. Different synthesis conditions were therefore assayed by varying the nature of the monomer and of the porogen, and the most promising MIP in terms of selective retention for CLD (extraction recovery close to 100%) was completely characterized by solid-phase extraction (repeatability of the extraction procedure, of the synthesis, and of the cartridge filling) in pure medium. The capacity of the MIP was determined at 0.13 $\mu\text{mol g}^{-1}$ of MIP. After application of a spiked bovine serum sample on the MIP, the selective retention was maintained (87 and 21%, respectively, on the MIP and on the corresponding non-imprinted polymer). Moreover, extraction on the MIP led to a cleaner extract compared to those issued from a conventional C18 sorbent.

Keywords: chlordecone; molecularly imprinted polymers; solid-phase extraction; LC-MS/MS; bovine serum



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1. Introduction

Chlordecone (CLD) is a non-aromatic organochlorine insecticide widely used between 1972 and 1993 in the French West Indies to protect banana plantations from black weevil [1]. CLD is known to be carcinogenic, mutagenic, and reprotoxic and is suspected of being an endocrine disrupter [2–4]. Even after 30 years, all environmental compartments in Martinique and Guadeloupe are largely contaminated, leading to the indirect contamination of locally reared animals (chickens, pigs, goats, and cattle) through unintentional soil ingestion [5]. It can be enzymatically metabolized in the livers of some mammals in chlordecol (CLD-OH) and eliminated through excretion in urine [1,6].

Regarding food safety and products of animal origin, market production is submitted to official controls: carcasses with a CLD concentration above 27 $\mu\text{g kg}^{-1}$ (mitigation measure) in perirenal fat are guaranteed, in respect to the maximum reference level (MRL of 20 $\mu\text{g kg}^{-1}$ [7]), to be removed from sale. Since correlations have been established between the CLD's concentration in perirenal fat and muscle (most consumed parts) or offals and serum [6,8], the question of pre-slaughter control of animals by monitoring CLD in relatively “non-invasive” biological fluids, such as serum, is raised. In the case of probable MRL violation, farm management could thus proceed to depuration under

controlled conditions or to change pasture area. This would have serious impact on both economic and food safety management thanks to these mitigation measures.

A rapid and sensitive analytical method based on gas chromatography coupled to electron capture dissociation (GC-ECD) leading to a limit of quantification (LOQ) close to $10 \mu\text{g kg}^{-1}$ was developed in the 1980s for the determination of CLD in food [9]. Today, GC is still used but coupled with mass spectrometry for its higher specificity, especially at low concentrations [10]. However, more recent studies use liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) preceded, or not, by an isotopic dilution for quantifying CLD in food, tissues, or biological fluids leading to an LOQ of only a few $\mu\text{g kg}^{-1}$ [11–13].

Regardless of the analytical methods used and the nature of the sample, after an optional grinding and homogenization of the sample, the sample preparation protocols are adapted from those of persistent organic pollutants. This includes numerous steps of liquid–liquid partition with binary mixtures composed mainly of a hydrophobic solvent (protic or not), such as hexane or dichloromethane (DCM), and of a small proportion of a polar one such as acetone [10,14] or a pressure liquid extraction with a mixture of hexane/DCM/methanol (MeOH), 5/2/1, v/v/v [15]. Then, an additional cleanup of the organic layer is performed with NaOH treatment and acidic lysis with sulfuric acid [10] or with a solid-phase extraction (SPE) step on Florisil® cartridges [15]. More recently, a method dedicated to the analyses of CLD in animal liver [11,12], feces, and urine of ewes [11,12], based on QuEChERS method, was validated. Concerning the extraction procedure for blood samples (serum [13] or plasma [16]), it is based on a slightly simpler protocol originally dedicated to the extraction of polychlorobiphenyls (PCB) [15]. Briefly, after denaturation of the proteins and hydrolysis of fat, thanks to addition of triethylamine and formic acid to the sample, the extract was subject to an SPE on C18.

A more selective treatment of samples based on molecularly imprinted polymers (MIPs) specifically designed for a target molecule [17] prior LC/MS analysis should help to significantly simplify the procedure and reduce the impact of matrix effects and, thus, help to increase the overall performance of the method. MIPs are synthetic polymeric materials possessing cavities specifically designed for a template molecule. Compared to other biomimetic materials (oligosorbents or immunosorbents that are based on aptamers or antibodies, respectively), MIPs have the advantage of being cheaper to produce, stable, and easily reusable [17]. Their efficiency has also been demonstrated for the extraction of different classes of compounds (pesticides, drugs, emerging contaminants, proteins, and natural products such as toxins) from various complex samples (environmental samples, food extracts, beverages, biological fluids, etc.) [17].

To our knowledge, the synthesis of MIP for CLD has never been described. The absence of amino acid groups or aromatic rings on CLD that favor the establishment of non-covalent interactions (hydrogen bonds or π – π interactions) between the template and the conventional monomers (acid, basic, or neutral), makes it difficult to synthesize a specific MIP for this molecule that is only characterized by numerous chloride groups. The synthesis of MIPs was already described for other organochloride compounds [18–25] that possess aromatic rings or sulphate groups [26–28] that may favor the establishment of π – π type interactions or other strong polar interactions during the synthesis. It was also described for non-aromatic organochloride compounds such as heptachlor [28–30], dieldrin [28], and lindane [31,32] to develop electrochemical sensors [27,30] or to carry out binding experiments in pure solvent [26,31] or as SPE sorbents [28,30]. For all these MIPs, the imprinting factor (IF) that corresponds to the ratio between the fixed amount of the target compound on the MIP and the non-imprinted polymer (NIP), synthesized in the same way but without introduction of the template molecule, was measured in pure solvent and gave rise to values lower than two. These IFs are low compared to the values reported in the literature for other molecules (usually higher than three) thus indicating a limited selectivity of the MIP [17,33]. The aim of this work was thus to synthesize a MIP for the selective extraction of CLD from complex samples and to integrate this selective

tool in the analytical procedure to improve its robustness for the determination of low levels of CLD in bovine serum which could eventually allow its use before the slaughter of animals. For this, different conditions of synthesis were investigated using CLD-OH as a template molecule and by varying the nature of the monomer and the porogen used. After a first screening in pure medium of the different MIP by studying in parallel the retention of CLD on MIPs and on a non-imprinted polymer, the MIP that gave the most promising results in terms of retention and selectivity for CLD in pure medium was characterized more completely. The repeatability of the extraction procedure of the synthesis and of the cartridge filling were studied and the capacity was determined. Finally, the performances of the developed MIP in terms of extraction yields and cleanup efficiency for bovine serum samples were compared to those of the commercial C18 silica sorbent.

2. Materials and Methods

2.1. Chemicals and Instrumentation

CLD (98%) and CLD-OH (98%) standards were purchased from Cluzeau Info Labo (Sainte-Foy-La-Grande, France). Working solutions were stored at 5 ± 3 °C. Bovine serum for MIP performance study was purchased from Fisher Scientific (Illkirch, France). The serum was checked to be CLD and CLD-OH free. Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), 4-vinylpyridine (4 VP), and formic acid (FA) were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France). Washed EGDMA, MAA, and 4 VP were distilled under vacuum in order to remove inhibitors. Azo-*N,N'*-bis-isobutyronitrile (AIBN) was purchased from Acros Organics (Noisy-le-Grand, France). HPLC-grade acetonitrile (ACN), methanol (MeOH), dichloromethane (DCM), chloroform, heptane, and toluene were supplied by Carlo Erba (Val de Reuil, France). High-purity water was dispensed by a Milli-Q purification system (Millipore, Saint Quentin en Yvelines, France).

2.2. Instrumentation and Analytical Conditions

The analyses were performed using a liquid chromatograph (UltiMate 3000[®], Thermo Scientific, Illkirch, France) coupled with a triple-stage quadrupole mass spectrometer (TSQ Quantum Access MAX, Thermo Scientific) equipped with a heated electrospray ionization source (HESI2). The chromatographic separation was performed on an Atlantis C18 column (Waters Corporation, 150 × 2.1 mm, 5 μm) heated at 40 °C in the isocratic mode with a mobile phase composed of H₂O/ACN/FA (39.9/60/0.1, v/v/v). The flow was set 0.3 mL min⁻¹, the injection volume at 5 μL, and the run time at 10 min. MS was operated in the negative ion mode with MRM detection using an electrospray voltage of 3500 V, a sheath gas and an ion sweep gas pressure of 40 and 2 psi, respectively. Nitrogen was used as the desolvation gas and argon as the collision gas at a pressure of 1.5 mTorr. Capillary and vaporizer temperatures were set at 395 and 277 °C, respectively, and tube lens offsets of 90 and 80 V were used for CLD and CLD-OH, respectively. As in previous studies, CLD was detected under the deprotonated hydrated form and, thus, the transition 506.7 → 426.7 (21 eV) was used to quantify it [11,13]. Regarding CLD-OH, as already reported by He et al. [34], the compound was not fragmented (even using high collision energy and the pseudo MRM transition 490.5 → 490.5 was used for quantification purpose). The calibration curves ($y = 26,163,761x$, $r^2 = 0.988$ and $y = 8,044,210x$, $r^2 = 0.997$ for CLD and CLD-OH, respectively) were linear over the concentration range of 50 ng L⁻¹ to 2.5 mg L⁻¹ for CLD and of 10 μg L⁻¹ to 5 mg L⁻¹ for CLD-OH.

2.3. Condition of Synthesis of Molecularly Imprinted Polymers

Four couples of MIP/NIPs (MIP/NIP 1–4) were synthesized using CLD-OH as a template molecule, EGDMA as a crosslinker, and different combinations of monomers and solvents (details in Table 1). The polymers were synthesized using the most commonly used molar ratio between the template, the monomer, and the crosslinker: 1/4/20 [33,35–38]. Briefly, 0.5 mmol of the template was mixed with 2 mmol of the monomer in 0.9 mL of porogen. The obtained mix was sonicated for 10 min. Then, 10 mmol of EGDMA

was added and the mixture was purged for 10 min on a gentle N₂ stream, 0.1 mmol of AIBN, as the initiator, was added and the polymerization mixture was placed at 54 °C in a water bath for 24 h. Each polymer was then crushed, ground automatically in a mixer (MIL MM 301, Retsch®, Haan, Germany), and sieved in a vibratory sieve shaker (Retsch®, Haan, Germany) to collect particles between 25 and 36 µm. A sedimentation step was achieved with 4 × 5 mL of MeOH/water (80/20, v/v) to remove the thinnest particles and then dried at room temperature overnight. Non-imprinted polymers were synthesized following the same procedure except that the template molecule was not introduced in the polymerization mixture.

Table 1. Composition of the polymerization mixture used for MIP/NIPs syntheses.

Name	Template (for MIPs Only)	Monomer	Porogen
MIP/NIP 1	CLD-OH	MAA	Chloroform
MIP/NIP 2	CLD-OH	4-VP	ACN
MIP/NIP 3	CLD-OH	4-VP	Toluene
MIP/NIP 4	CLD-OH	4-VP	Chloroform

According to the capacity previously reported for MIPs developed for compounds [17], 35 mg of MIP or NIP particles were weighted and introduced in 1 mL disposable polypropylene cartridges (Sigma–Aldrich, Saint Quentin Fallavier, France) beforehand equipped with one polyethylene frit (Sigma–Aldrich, Saint Quentin Fallavier, France). A second polyethylene frit was added to the top of the cartridge. The MIP/NIP cartridges were then washed with MeOH to remove the template molecule until the template molecule could no longer be detected in the washing fraction by LC/MS (instrumental LOQ allowed us to detect a leak representing only 0.042% of the initial amount of template molecule introduced during the synthesis process).

2.4. Preliminary Evaluation of the MIP/NIPs by Solid-Phase Extraction in Pure Organic Media

The first extraction procedure was performed on each coupled MIP/NIP to evaluate the different MIP/NIPs synthesized in terms of retention capacity and selectivity. All solid-phase extraction steps were performed manually, and the flow rate was set at 1 droplet per second (approximately 1 mL min⁻¹). The MIP/NIP cartridges were first conditioned with 1 mL of the same solvent as the one used as porogen for the MIP synthesis. This step was followed by the percolation of 1 mL of the same solvent spiked with CLD and CLD-OH at a concentration level of 200 µg L⁻¹. Then, five washing steps were performed with 1 mL of a mixture of the solvent used for percolation and methanol with increasing elution strength (0, 10, 20, 30, and 40% of volume, respectively, for the five washing fractions). Finally, three fractions of 1 mL of methanol were used to ensure complete elution of CLD and CLD-OH from the support. All the fractions were evaporated under N₂ stream and resuspended in 100 µL of H₂O/ACN/FA (39.9/60/0.1, v/v/v) before injection into LC/MS.

2.5. Characterization of the Most Promising MIP/NIP (MIP/NIP 2) in Pure Organic Media

According to the preliminary results and in order to reduce the number of washing and elution steps, the following procedure was applied to the MIP/NIP 2: The conditioning was performed with 1 mL of ACN, followed by the percolation of 1 mL of ACN spiked with 200 µg L⁻¹ of CLD and CLD-OH and by a washing with 1.5 mL of a mixture ACN/MeOH, 95/5, v/v. Finally, the elution was performed in one step with 3 mL of MeOH. All the fractions were evaporated under N₂ stream and resuspended in 100 µL of H₂O/ACN/FA (39.9/60/0.1, v/v/v) before injection into LC/MS.

The capacity of the MIP 2 was studied by percolating 1 mL of ACN spiked at 16 different levels of concentration of CLD (ranging from 5 µg L⁻¹ to 3 mg L⁻¹) through the cartridge and by applying the same washing and elution conditions as described above.

Similar experiments were carried out on the NIP. For the higher concentration levels, the resuspension volume of the elution fractions after evaporation to dryness was adapted in order to stay in the linearity range of the LC/MS calibration curves.

2.6. Extraction Procedure Applied to Bovine Serum

A simple precipitation of proteins with 3 volumes of ACN was performed on 3 mL of bovine serum spiked at $10 \mu\text{g L}^{-1}$, followed by a centrifugation step at 4500 rpm for 5 min. The supernatant was recovered, evaporated under N_2 stream, and resuspended in 3 mL of pure ACN for further extraction on MIP/NIP 2 or of water for extraction on a Sep-Pack C18, 1 cc, 50 mg (Waters).

For MIP/NIP 2, 0.5 mL of the serum extract was then percolated with the optimized extraction procedure described in Section 2.5. For the Sep-Pack C18, 1 cc, 50 mg (Waters), the extraction procedure was adapted from the only publication that focuses on the quantification of CLD in serum (human) [13]. Briefly, after conditioning the sorbent with 0.5 mL of ACN and 1 mL of water, 0.5 mL of the serum extract was percolated. A first washing step with 2 mL of water was followed by a drying under vacuum for 75 min of the sorbent and by a second washing with 1 mL of hexane. Finally, after a second drying under vacuum during 5 min, CLD was eluted thanks to 1 mL of ACN. The second washing fraction and the elution fraction were evaporated to dryness under an N_2 stream and resuspended in 100 μL of mobile phase before injection into LC/MS.

2.7. Evaluation of the Matrix Effects during LC/MS-MS Analysis

Matrix effects were evaluated by comparing the slopes of three calibration curves constructed in a pure medium ($\text{H}_2\text{O}/\text{ACN}/\text{FA}$, 39.9/60/0.1, v/v/v) in the elution fraction resulting from MIP 2 applied to serum extract and in the elution fraction obtained by applying the SPE procedure on the Sep-Pack C18. For this, a blank serum extract (0.5 mL) was percolated 3 times on each support (MIP and Sep-Pack C18) according to the procedure described in Section 2.6, and the resulting elution fraction for each support was equally divided in two before evaporation to dryness under an N_2 stream. The residue was reconstituted in 50 μL of the mobile phase containing CLD at 6 concentrations levels (0, 1, 5, 20, 100, and 1000 $\mu\text{g L}^{-1}$) before injection into LC/MS.

3. Results and Discussion

3.1. Screening of the Synthesis Conditions

3.1.1. Choice of the Synthesis Conditions

The template molecule plays a crucial role in the formation of cavities that will be further used to selectively trap the targeted compound in real samples. In all the previously cited studies related to the development of MIP for organochloride, the targeted compound was used as the template molecule for the synthesis. However, our group demonstrated that a slight release of the template molecule could occur ($<4.7 \times 10^{-5}\%$ of quantity introduced during the synthesis process) during further extraction and, thus, led to a false positive response in the case of targeting compounds at the ultra-trace level in complex matrices [38]. In order to circumvent this possible issue and considering the planned future applications (i.e., CLD present in serum), CLD-OH, the main metabolite of CLD, was used for the first time as a template molecule.

Four conditions of synthesis were thus studied (see Table 1), all of them were based, for the first time, on the use of the dummy template (i.e., CLD-OH) with commonly used monomers MAA or 4-VP and in different porogenic solvents. For each synthesis, an NIP was synthesized with the same reagents but in the absence of a template molecule to provide a control support to be used to evaluate non-specific interactions. Among the four conditions of synthesis tested, MIP/NIP 3 and 4 led to inhomogeneous polymers. Thus, only the MIPs resulting from the first two conditions of synthesis allowed for us to obtain homogeneous polymers that were fully characterized by solid-phase extraction of CLD and the template CLD-OH in pure media.

3.1.2. Evaluation of the MIP/NIPs by Solid-Phase Extraction in Pure Organic Solvents

The first SPE procedure that consisted of the percolation of a solution constituted by the solvent used for the synthesis of the MIP/NIPs spiked with the target molecule and the template (i.e., CLD and CLD-OH) was therefore applied to MIP/NIPs 1 and 2. Indeed, the use of the solvent implied in the synthesis as a percolation solvent should favor the retention of the targeted compounds on the MIP by interactions similar to those involved during the synthesis.

For MIP/NIP 1, synthesized with MAA and EGDMA in chloroform, the percolation of spiked chloroform led to a significant loss of CLD and CLD-OH from both MIP and NIP (loss ranging from 45 to 65%), with the remainder lost in the first washing step. In order to improve the retention on MIP 1, the percolation step was then performed in heptane (i.e., a less polar solvent) and led to (i) the total retention of CLD both on MIP and on NIP and a (ii) nearly 86% loss of CLD-OH from NIP 1. The washing step performed with 1 mL of pure heptane led to a significant loss of CLD-OH (71%) from the MIP while maintaining CLD retained on both the MIP and the NIP. MIP/NIP 1 was thus found to be highly retentive for CLD but without any selectivity as a similar retention was obtained on MIP and on NIP and led to a low retention for CLD-OH.

Concerning MIP/NIP 2, the percolation of spiked ACN led to a strong retention of CLD on both sorbents, while a loss of 68% of CLD-OH was observed on NIP 2. During the next two washing steps (first with ACN and the second one with ACN/MeOH, 90/10, v/v), approximately 76% of the CLD was lost on NIP 2, while only 25% was lost on MIP 2, indicating a higher retention of CLD on MIP than on NIP. Regarding CLDOH, a loss of approximately 30% was observed from the first washing step on both MIP and NIP (see complete extraction profile in Supplementary Materials Figure S1). Even if the retention of CLD-OH was lower than that of CLD, this compound was still retained selectively on the MIP. In order to improve the retention of both compounds on the MIP while maintaining selectivity, i.e., a low retention on the NIP, a simplified extraction procedure, including a single washing step with a lower eluting strength, was tested. This included a single washing step with 1.5 mL of an ACN/MeOH mixture, 95/5, v/v, followed immediately by the elution step with 3 mL of MeOH. This extraction procedure allowed for (i) the retention of 90% of the CLD on the MIP until the elution step, while 96% of this compound was lost during the washing step on the NIP; (ii) the loss of 86% of the CLD-OH during the percolation step on the NIP and the recovery of 71 and 30% of CLD-OH, respectively, in the washing and elution fractions issued from the MIP. In view of its ability to selectively retain CLD and CLD-OH, this coupled MIP/NIP 2 was thus selected for the rest of the study.

3.2. Characterization of the Most Promising MIP/NIPs (MIP/NIP 2) in Pure Organic Media

3.2.1. Repeatability of the Extraction Procedure

After this preliminary evaluation, the simplified extraction procedure was applied in triplicate on the MIP/NIP 2. As seen in Figure 1, CLD was fully recovered in the elution fraction of the MIP with a standard deviation (SD) of 12%, showing the good repeatability of the extraction procedure. In addition, almost all of the CLD was lost during the percolation (11%) and washing steps (90%) on the NIP, with only 1% being retained until the elution step. This demonstrates the high selectivity of the retention of CLD observed on the MIP. Even if CLD-OH was the template molecule used during the synthesis of the MIP, it was less retained than CLD on the MIP. Indeed, CLD-OH was mostly lost during the washing step (74%). However, CLD-OH was, indeed, selectively retained on the MIP in a repeatable manner (SD < 5%) since its retention on the NIP was much lower (79% loss during the percolation step).

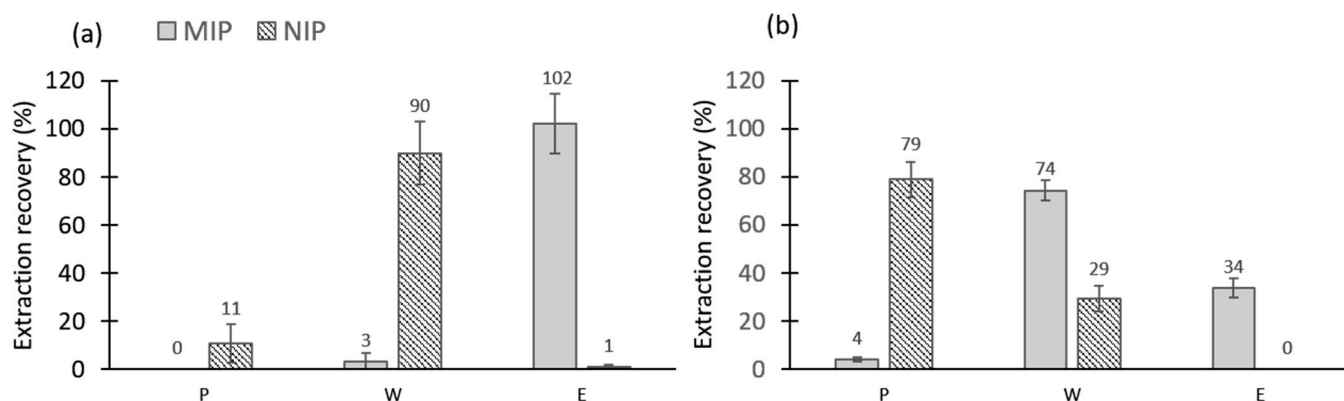


Figure 1. Repeatability of the extraction procedure obtained when percolating: (a) CLD and (b) CLD-OH in pure media on the MIP/NIP 2 ($n = 3$). Percolation (P): 1 mL ACN spiked at $200 \mu\text{g L}^{-1}$ with each compound; washing (W): 1 mL of ACN/MeOH, 95/5, v/v; Elution (E): 3 mL MeOH.

3.2.2. Repeatability of the Cartridge Filling

The objective being, in the long term, to apply these MIPs to a large number of samples, the preparation of many cartridges may be necessary, also involving numerous independent syntheses. Therefore, the repeatability of the synthesis and of the cartridge filling was assessed by focusing first on the latter point. For this, a second independent syntheses of MIP/NIP 2 was performed, and three cartridges were prepared. Then, extraction procedures were applied in triplicate on each of these three new cartridges ($n = 9$). A one-way ANOVA test ($\alpha = 0.05$) was thus performed on the extraction recovery in each fraction on newly synthesized MIP 2 and on NIP 2 and demonstrated that there were no significant differences among the results obtained on the three couples of cartridges issued for this new synthesis. This clearly demonstrates the repeatability of the cartridges' filling. Moreover, the elution profiles obtained on the MIP and on the NIP for CLD and CLD-OH (Figure 2) were similar to those obtained using a single cartridge filled with the polymers issued for the first synthesis (Figure 1). However, the newly synthesized NIP seemed to be slightly more retentive for CLD (17% of the CLD was retained until the elution step) than the previous one. Therefore, the repeatability of the synthesis was assessed.

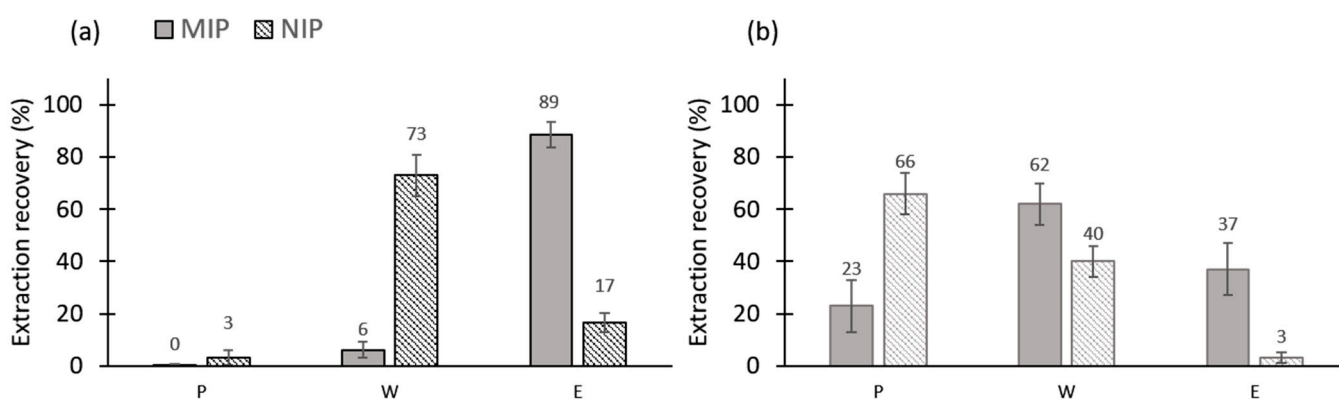


Figure 2. Extraction profiles obtained when percolating in triplicate 1 mL ACN spiked at $200 \mu\text{g L}^{-1}$ with (a) CLD and (b) CLD-OH on three different cartridges issued for the second independent synthesis of the MIP/NIP 2 ($n = 9$). The extraction procedure was the same as in Figure 1.

3.2.3. Reproducibility of the MIP/NIP 2 Synthesis

To assess the reproducibility of the synthesis, a third independent syntheses of MIP/NIP 2 was conducted. Then, the extraction procedure was applied in triplicate on three cartridges ($n = 9$), and each cartridge was filled with MIP particles resulting from

the three independent syntheses (Figure 3). The one-way ANOVA test ($\alpha = 0.05$) again demonstrated that there were no significant differences between the results obtained by the three cartridges issued by the three independent syntheses. This showed the good reproducibility of the synthesis procedure. In addition, RSD values of 11.6 and 6% for CLD and CLD-OH, respectively, were obtained for extraction recovery in the elution fraction of MIP 2. It demonstrated a good global reproducibility of the extraction procedure in pure medium (three procedures performed on three cartridges issued by three independent syntheses).

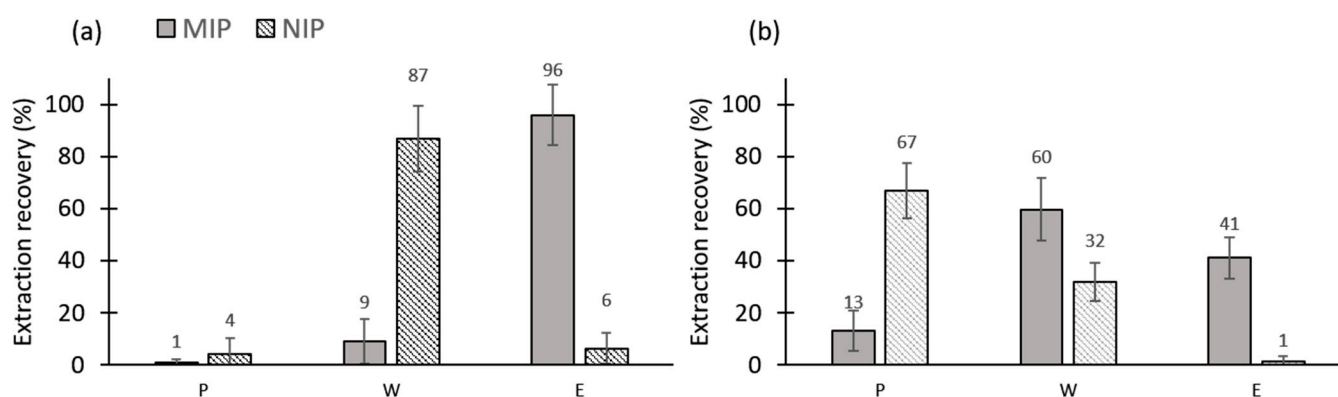


Figure 3. Extraction profiles obtained when percolating in triplicate 1 mL ACN spiked at $200 \mu\text{g L}^{-1}$ with (a) CLD and (b) CLD-OH on three cartridges containing MIP particles issued from three independent syntheses of the MIP/NIP 2 ($n = 9$). The extraction procedure was the same as in Figure 1.

3.2.4. Capacity of the MIP 2

In order to complete the characterization of the MIP/NIP 2 in pure medium, its capacity was evaluated by percolating increasing amounts of the targeted compound CLD. The capacity corresponds to the maximum amount of a targeted compound that can be retained by the MIP with a constant extraction recovery in the elution fraction. Figure 4 represents the amount of CLD determined in the elution fraction of MIP 2 and NIP 2 as a function of the amount percolated on the corresponding sorbent. The curve obtained for MIP 2 presented a linear part from 5 ng to $2.2 \mu\text{g}$ of CLD percolated on 35 mg of MIP. It demonstrated the linearity of the response (96% of extraction recovery in the elution fraction) in this range. Then, the slope of the curve strongly decreased and the intersection of both parts of the curve allowed for the determination of the capacity (saturation of the specific cavities of the MIP) at $62.8 \mu\text{g/g}$, i.e., $0.13 \mu\text{mol g}^{-1}$. This value was slightly lower than the capacity values generally reported after the radical polymerization of organic monomers ($1\text{--}40 \mu\text{mol/g}$) [39]. However, this capacity was sufficient to apply MIP to naturally contaminated serum samples. The slope of 4% observed for NIP 2 corresponded to the retention of CLD by non-specific interactions and was consistent with the mean extraction recoveries in the elution fraction previously obtained for the percolation of samples spiked at $200 \mu\text{g L}^{-1}$ in pure medium ($7 \pm 6\%$). This slope was also similar to the one of the second part of the curve obtained for the percolation of large amounts of CLD on MIP 2, which is explained by the fact that, once the capacity was reached, i.e., the cavities were saturated, the retention of the excess of CLD was also performed by non-specific interactions.

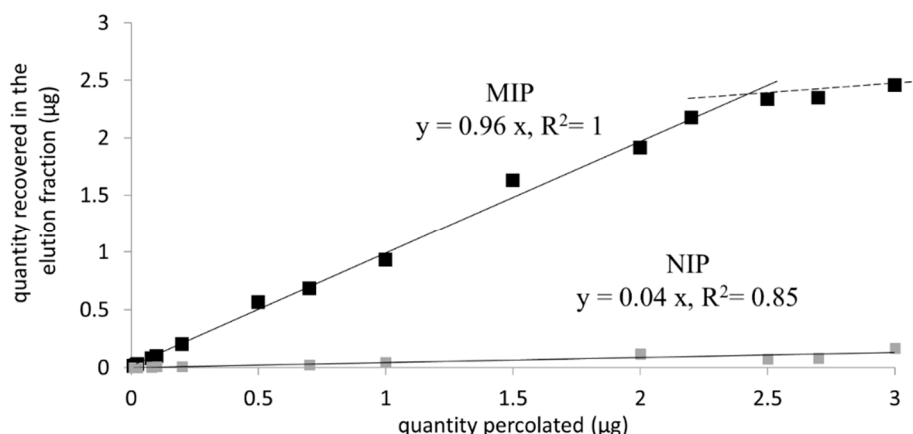


Figure 4. Amount of CLD (µg) quantified in the elution fraction as a function of the amount of CLD percolated on MIP/NIP 2. The extraction procedure was the same as in Figure 1, except that the percolation solution was spiked with an increasing amount of CLD.

3.3. Optimized Extraction Procedure Applied to Bovine Serum

3.3.1. Retention of CLD in Bovine’s Serum

It has been shown that CLD is present in the plasma of contaminated mammals [6]. However, the maximum residual concentration of 20 µg kg⁻¹ only applies to animals tissues intended for human consumption [6,8]. Given the objective of this study, which was to allow for a pre-slaughter control of bovine, the previously optimized procedure was thus applied to bovine serum spiked with CLD at a level of concentration set arbitrarily at 10 µg L⁻¹. The bovine serum was submitted to a simple precipitation of proteins with 3 volumes of ACN, and the final extract diluted in ACN (equivalent 0.5 mL of bovine serum) was percolated on MIP 2 and NIP 2. The resulting recovery yields in the washing and the elution fractions are reported in Table 2. It should be mentioned that the same procedure applied to a non-spiked serum sample did not allow for the detection of CLD. The high recovery of CLD obtained for the elution fraction of MIP 2 was very close to those obtained in pure media, and the selectivity of the extraction procedure was maintained, as only 21% of CLD was recovered in the elution fraction of NIP 2. Extraction yields obtained for this biological sample extract were also repeatable, as the SD values were lower than 8% in all of the analyzed fractions.

Table 2. Extraction recoveries of CLD obtained by percolating 0.5 mL of bovine serum spiked at 10 µg L⁻¹ on MIP, NIP, and Sep-Pack C18 sorbent (% ± SD values, n = 3).

	Washing Fraction	Elution Fraction
MIP 2	18 ± 7	87 ± 6
NIP 2	58 ± 8	21 ± 5
Sep-Pack C18	0	76 ± 10

3.3.2. Comparison of Performances with a Conventional Sorbent and Evaluation of Matrix Effects

Performances of the MIP in terms of extraction recovery yields and cleanup efficiency were also compared to that of Sep-Pack C18 sorbent by percolating 0.5 mL of serum extract treated in the same way than for the MIP (protein precipitation with 3 volumes of ACN), except that the resuspension of the dry extract was performed in water to be consistent with the condition of percolation previously reported for this sorbent [13]. It is interesting to note that the use of immiscible solvent during the Sep-Pack C18 extraction procedure requires two intermediate drying steps of the sorbent which turned out to be particularly time consuming and led to multiplying by 3.5 the time needed to perform the complete extraction procedure. Extraction yields of 76 ± 10% were obtained (Table 2) with the C18

silica sorbent, slightly lower than those obtained with the MIP 2, but the difference between both values was not significant (one-way ANOVA $\alpha = 0.05$). To achieve the comparison, matrix effects that could affect the quantification by LC-MS after treatment of serum on MIP or on Sep-Pack C18 were also monitored. For this purpose, a blank serum was extracted on each of the sorbents, and the extracts issued of their respective elution fraction were spiked at six concentration levels just before analysis by LC/MS. Both extraction methods led to a similar decrease (student tests with a confidence level of 95%, $\alpha = 0.05$) in the slope of the calibration curves, a decrease of 23 and 17% for MIP and Sep-Pack C18, respectively (calibrations curves are provided in Supplementary Materials Figure S2). However, the elution fraction resulting from the use of the Sep-Pack C18 was more complex than the one issued for the MIP, as indicated by the higher base line noise observed using the C18 than using the MIP, and by the presence of many peaks on the base peak LC-MS chromatogram in the scan mode (m/z 100–1000) when analyzing the C18 silica extract (Figure 5a). The improved extract purification through the use of MIP as compared to the Sep-Pack C18 reduced fouling of the LC/MS system and, therefore, could limit its maintenance and led to an increase in the overall instrument lifetime. Moreover, the S/N ratio obtained for the peak of CLD (retention time = 5.2 min) in the MRM mode for its quantification in the elution fraction was four times lower using conventional sorbent (S/N = 5707) (Figure 5b) than using the MIP (S/N = 22,546) (Figure 5c).

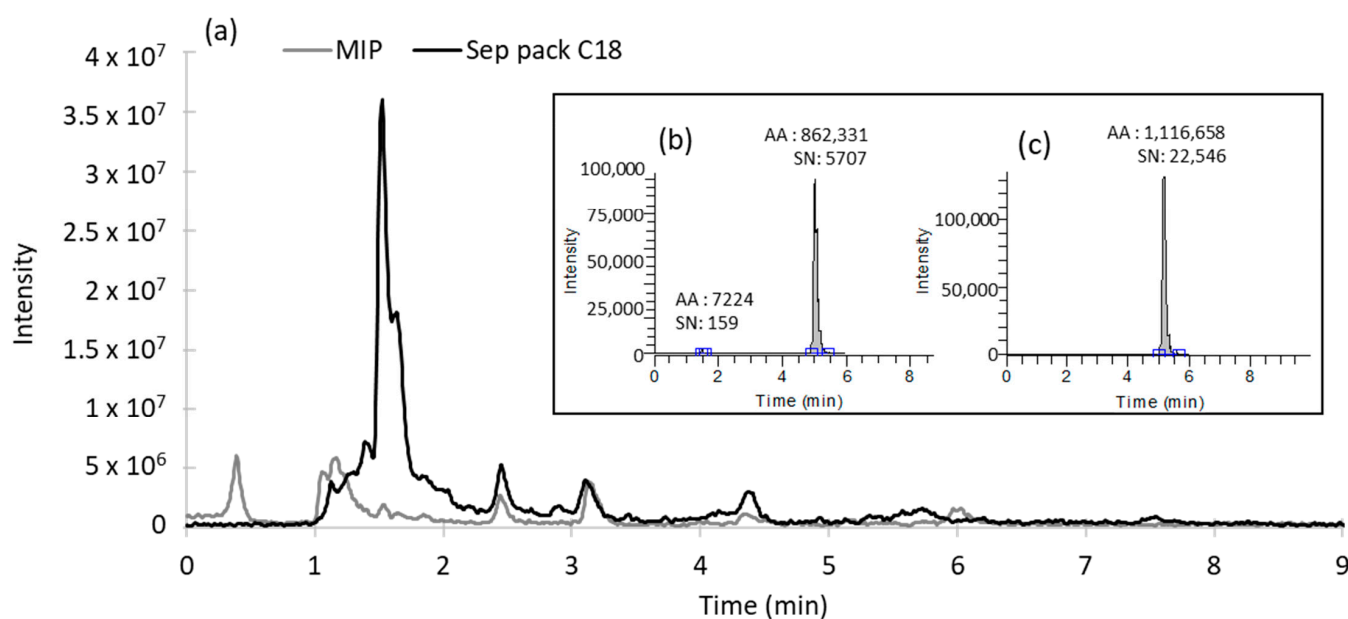


Figure 5. (a) Base peak LC-MS-chromatograms (scan mode, m/z 100–1000) corresponding to the elution fraction obtained after the percolation of 0.5 mL of the serum's extract spiked at $10 \mu\text{g L}^{-1}$ with CLD on MIP (gray line) and on the Sep-Pack C18 (black line). The insert corresponds to the MRM chromatograms for CLD ($506.7 \rightarrow 426.7$) corresponding to the elution fraction issued by the Sep-Pack C18's sorbent (b) and MIP (c).

The LOQ of the global analytical procedure, including extraction on the MIP and on the Sep-Pack C18, was thus estimated at a concentration level of 4.4 and 17.5 ng L^{-1} , respectively. Previous studies related to the development of analytical methods for the analysis of CLD in plasma and human serum reported LOQs of 750 [16] and 25 ng L^{-1} , respectively [13]. In addition to the simplification of the serum treatment step (a single ACN precipitation step in this study instead of a two-step procedure, including the denaturation of the proteins followed by the hydrolysis of fat, previously used [13,16]), the use of the MIP as a selective tool during the sample treatment allowed us to decrease the LOQ of the global analytical procedure by a factor ranging from 5 to 180.

4. Conclusions

Among the synthesis conditions tested, the most promising MIP was synthesized with CLD-OH as the dummy template, 4-VP as the monomer, EGDMA as the crosslinker with a molar ratio of 1/4/20, and ACN as the porogen. The repeatability of the extraction procedure, the cartridge filling, and the synthesis procedure were verified. Then, the performances were further evaluated thanks to a spiked bovine serum sample, and it demonstrated a more efficient cleanup of the sample compared to a conventional Sep-Pack C18 sorbent, thus improving the global limit of quantification of CLD in bovine serum by a factor of five. Altogether, it tends to show that further investigations, in particular on the extraction procedure, could allow for the extension of the field of application of the imprinted polymer to other samples of animal origin such as urine or food samples.

5. Patents

Bosman, P.; Combès, A.; Lambert, M.; Lavison Bompard, G.; Pichon, V. Polymère à empreintes moléculaires, et son utilisation dans la quantification d'un pesticide dans un échantillon. FR2108363. 30/07/2021.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/separations8120237/s1>, Figure S1: Extraction profile obtained when percolating (a) CLD and (b) CLD-OH in pure media on the MIP/NIP 2. Percolation: 1 mL of 200 $\mu\text{g L}^{-1}$ of each compound in ACN; washing: 1 mL of ACN, 1 mL of ACN/MeOH, 90/10, v/v; 1 mL of ACN/MeOH, 80/20, v/v, 1 mL of ACN/MeOH, 70/30, v/v, 1 mL of ACN/MeOH, 60/40, v/v, Elution: 3 mL MeOH; Figure S2: Calibration curves for CLD obtained for spiked pure water (square), spiked extract of serum's sample obtained after SPE on an MIP (diamond) or on a Sep-Pack C18 (triangle).

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References

1. Jondreville, C.; Lavigne, A.; Jurjanz, S.; Dalibard, C.; Liabeuf, J.-M.; Clostre, F.; Lesueur-Jannoyer, M. Contamination of Free-Range Ducks by Chlordecone in Martinique (French West Indies): A Field Study. *Sci. Total Environ.* **2014**, *493*, 336–341. [[CrossRef](#)]
2. Boucher, O.; Simard, M.-N.; Muckle, G.; Rouget, F.; Kadhel, P.; Bataille, H.; Chajès, V.; Dallaire, R.; Monfort, C.; Thomé, J.-P.; et al. Exposure to an Organochlorine Pesticide (Chlordecone) and Development of 18-Month-Old Infants. *Neurotoxicology* **2013**, *35*, 162–168. [[CrossRef](#)]
3. Cordier, S.; Bouquet, E.; Warembourg, C.; Massart, C.; Rouget, F.; Kadhel, P.; Bataille, H.; Monfort, C.; Boucher, O.; Muckle, G.; et al. Perinatal Exposure to Chlordecone, Thyroid Hormone Status and Neurodevelopment in Infants: The Timoun Cohort Study in Guadeloupe (French West Indies). *Environ. Res.* **2015**, *138*, 271–278. [[CrossRef](#)] [[PubMed](#)]
4. Hervé, D.; Costet, N.; Kadhel, P.; Rouget, F.; Monfort, C.; Thomé, J.-P.; Multigner, L.; Cordier, S. Prenatal Exposure to Chlordecone, Gestational Weight Gain, and Birth Weight in a Guadeloupean Birth Cohort. *Environ. Res.* **2016**, *151*, 436–444. [[CrossRef](#)]
5. Fournier, A.; Feidt, C.; Lastel, M.-L.; Archimede, H.; Thome, J.-P.; Mahieu, M.; Rychen, G. Toxicokinetics of Chlordecone in Goats: Implications for Risk Management in French West Indies. *Chemosphere* **2017**, *171*, 564–570. [[CrossRef](#)] [[PubMed](#)]
6. Fourcot, A.; Feidt, C.; Bousquet-Mélou, A.; Ferran, A.A.; Gourdine, J.L.; Bructer, M.; Joaquim-Justo, C.; Rychen, G.; Fournier, A. Modeling Chlordecone Toxicokinetics Data in Growing Pigs Using a Nonlinear Mixed-Effects Approach. *Chemosphere* **2020**, *250*, 126151. [[CrossRef](#)]
7. EURL | Residues of Pesticides | Pesticide EU-MRLs Database. Available online: https://www.eurl-pesticides.eu/docs/public/tmpl/article.asp?LabID=100&CntID=649&Theme_ID=1&Pdf=False&Lang=EN (accessed on 26 October 2021).

8. NOTE ANSES on the Establishment of a Maximum Residue Limit for Chlordecone in Fat for Meat Products. *Saisine n°2018-SA-0202*. Available online: <https://www.anses.fr/fr/system/files/ERCA2018SA0265.pdf> (accessed on 1 December 2021).
9. Meijs, A.W.H.M.; Ernst, G.F. Rapid Gas Chromatographic Method for the Determination of Kepone in the Eel. *J. Chromatogr. A* **1979**, *171*, 486–489. [[CrossRef](#)]
10. Bordet, F.; Thieffine, A.; Mallet, J.; Heraud, F.; Bateau, A.; Inthavong, D. In-House Validation for Analytical Methods and Quality Control for Risk Evaluation of Chlordecone in Food. *Null* **2007**, *87*, 985–998. [[CrossRef](#)]
11. Saint-Hilaire, M.; Bertin, T.; Inthavong, C.; Lavison-Bompard, G.; Guérin, T.; Fournier, A.; Feidt, C.; Rychen, G.; Parinet, J. Validation of Analytical Methods for Chlordecone and Its Metabolites in the Urine and Feces of Ewes. *J. Chromatogr. B* **2018**, *1093–1094*, 66–76. [[CrossRef](#)] [[PubMed](#)]
12. Saint-Hilaire, M.; Inthavong, C.; Bertin, T.; Lavison-Bompard, G.; Guérin, T.; Fournier, A.; Feidt, C.; Rychen, G.; Parinet, J. Development and Validation of an HPLC-MS/MS Method with QuEChERS Extraction Using Isotopic Dilution to Simultaneously Analyze Chlordecone and Chlordecol in Animal Livers. *Food Chem.* **2018**, *252*, 147–153. [[CrossRef](#)]
13. Bichon, E.; Guiffard, I.; Vénisseau, A.; Marchand, P.; Antignac, J.-P.; Le Bizec, B. Ultra-Trace Quantification Method for Chlordecone in Human Fluids and Tissues. *J. Chromatogr. A* **2015**, *1408*, 169–177. [[CrossRef](#)] [[PubMed](#)]
14. Woignier, T.; Fernandes, P.; Jannoyer-Lesueur, M.; Soler, A. Sequestration of Chlordecone in the Porous Structure of an Andosol and Effects of Added Organic Matter: An Alternative to Decontamination. *Eur. J. Soil Sci.* **2012**, *63*, 717–723. [[CrossRef](#)]
15. Debier, C.; Pomeroy, P.P.; Dupont, C.; Joiris, C.; Comblin, V.; Boulengé, E.L.; Larondelle, Y.; Thomé, J.-P. Dynamics of PCB Transfer from Mother to Pup during Lactation in UK Grey Seals *Halichoerus Grypus*: Differences in PCB Profile between Compartments of Transfer and Changes during the Lactation Period. *Mar. Ecol. Prog. Ser.* **2003**, *247*, 249–256. [[CrossRef](#)]
16. Multigner, L.; Ndong, J.R.; Giusti, A.; Romana, M.; Delacroix-Maillard, H.; Cordier, S.; Jégou, B.; Thome, J.P.; Blanchet, P. Chlordecone Exposure and Risk of Prostate Cancer. *JCO* **2010**, *28*, 3457–3462. [[CrossRef](#)] [[PubMed](#)]
17. Pichon, V.; Delaunay, N.; Combès, A. Sample Preparation Using Molecularly Imprinted Polymers. *Anal. Chem.* **2020**, *92*, 16–33. [[CrossRef](#)] [[PubMed](#)]
18. Du, X.; Lin, S.; Gan, N.; Chen, X.; Cao, Y.; Li, T.; Zhan, P. Multi-Walled Carbon Nanotube Modified Dummy-Template Magnetic Molecularly Imprinted Microspheres as Solid-Phase Extraction Material for the Determination of Polychlorinated Biphenyls in Fish. *J. Sep. Sci.* **2014**, *37*, 1591–1600. [[CrossRef](#)]
19. Xu, X.; Liang, S. Molecularly Imprinted Solid-Phase Extraction Method for the Gas Chromatographic Analysis of Organochlorine Fungicides in Ginseng. *J. Sep. Sci.* **2019**, *42*, 1393–1403. [[CrossRef](#)]
20. Wang, H.; Yan, H.; Qiu, M.; Qiao, J.; Yang, G. Determination of Dicofol in Aquatic Products Using Molecularly Imprinted Solid-Phase Extraction Coupled with GC-ECD Detection. *Talanta* **2011**, *85*, 2100–2105. [[CrossRef](#)]
21. Yan, H.; Sun, N.; Han, Y.; Yang, C.; Wang, M.; Wu, R. Ionic Liquid-Mediated Molecularly Imprinted Solid-Phase Extraction Coupled with Gas Chromatography-Electron Capture Detector for Rapid Screening of Dicofol in Vegetables. *J. Chromatogr. A* **2013**, *1307*, 21–26. [[CrossRef](#)]
22. Yan, H.; Yang, C.; Sun, Y.; Row, K.H. Ionic Liquid Molecularly Imprinted Polymers for Application in Pipette-Tip Solid-Phase Extraction Coupled with Gas Chromatography for Rapid Screening of Dicofol in Celery. *J. Chromatogr. A* **2014**, *1361*, 53–59. [[CrossRef](#)]
23. Qiao, F.; Gao, M.; Yan, H. Molecularly Imprinted Ionic Liquid Magnetic Microspheres for the Rapid Isolation of Organochlorine Pesticides in Environmental Water. *J. Sep. Sci.* **2016**, *39*, 1310–1315. [[CrossRef](#)] [[PubMed](#)]
24. Yang, C.; Lv, T.; Yan, H.; Wu, G.; Li, H. Glyoxal-Urea-Formaldehyde Molecularly Imprinted Resin as Pipette Tip Solid-Phase Extraction Adsorbent for Selective Screening of Organochlorine Pesticides in Spinach. *J. Agric. Food Chem.* **2015**, *63*, 9650–9656. [[CrossRef](#)] [[PubMed](#)]
25. Gao, X.; Pan, M.; Fang, G.; Jing, W.; He, S.; Wang, S. An Ionic Liquid Modified Dummy Molecularly Imprinted Polymer as a Solid-Phase Extraction Material for the Simultaneous Determination of Nine Organochlorine Pesticides in Environmental and Food Samples. *Anal. Methods* **2013**, *5*, 6128–6134. [[CrossRef](#)]
26. Shaikh, H.; Memon, N.; Bhangar, M.I.; Nizamani, S.M.; Denizli, A. Core-Shell Molecularly Imprinted Polymer-Based Solid-Phase Microextraction Fiber for Ultra Trace Analysis of Endosulfan I and II in Real Aqueous Matrix through Gas Chromatography-Micro Electron Capture Detector. *J. Chromatogr. A* **2014**, *1337*, 179–187. [[CrossRef](#)]
27. Zuo, H.G.; Yang, H.; Zhu, J.X.; Ding, Y. Preparation of a Novel RAM-MIP for Selective Solid-Phase Extraction and Gas Chromatography Determination of Heptachlor, Endosulfan and Their Metabolite Residues in Pork. *Anal. Methods* **2017**, *9*, 6009–6018. [[CrossRef](#)]
28. Silverio, O.V.; So, R.C.; Elnar, K.J.S.; Malapit, C.A.; Nepomuceno, M.C.M. Development of Dieldrin, Endosulfan, and Hexachlorobenzene-Imprinted Polymers for Dye-Displacement Array Sensing. *J. Appl. Polym. Sci.* **2017**, *134*. [[CrossRef](#)]
29. Singh, K.; Balasubramanian, S.; Rani, B.E.A. Computational and Experimental Studies of Molecularly Imprinted Polymers for Organochlorine Pesticides Heptachlor and DDT. *Curr. Anal. Chem.* **2012**, *8*, 562–568. [[CrossRef](#)]
30. Singh, K.; Pasha, A.; Rani, B.A. Preparation of Molecularly Imprinted Polymers for Heptachlor: An Organochlorine Pesticide. *Chron. Young Sci.* **2013**, *4*, 46.
31. Anirudhan, T.S.; Alexander, S. Design and Fabrication of Molecularly Imprinted Polymer-Based Potentiometric Sensor from the Surface Modified Multiwalled Carbon Nanotube for the Determination of Lindane (γ -Hexachlorocyclohexane), an Organochlorine Pesticide. *Biosens. Bioelectron.* **2015**, *64*, 586–593. [[CrossRef](#)]

32. Pelin Böke, C.; Karaman, O.; Medetalibeyoglu, H.; Karaman, C.; Atar, N.; Lütü Yola, M. A New Approach for Electrochemical Detection of Organochlorine Compound Lindane: Development of Molecular Imprinting Polymer with Polyoxometalate/Carbon Nitride Nanotubes Composite and Validation. *Microchem. J.* **2020**, *157*, 105012. [[CrossRef](#)]
33. Pichon, V. Selective Sample Treatment Using Molecularly Imprinted Polymers. *J. Chromatogr. A* **2007**, *1152*, 41–53. [[CrossRef](#)] [[PubMed](#)]
34. He, D.; Xia, B.; Liu, J.; Ding, L.; Zhou, Y. Determination of Kepone and Its Metabolite in Water and Soil by High-Performance Liquid Chromatography–Mass Spectrometry. *Anal. Lett.* **2015**, *48*, 1–8. [[CrossRef](#)]
35. Thibert, V.; Legeay, P.; Chapuis-Hugon, F.; Pichon, V. Synthesis and Characterization of Molecularly Imprinted Polymers for the Selective Extraction of Cocaine and Its Metabolite Benzoylcegonine from Hair Extract before LC–MS Analysis. *Talanta* **2012**, *88*, 412–419. [[CrossRef](#)]
36. Boulanouar, S.; Combès, A.; Mezzache, S.; Pichon, V. Synthesis and Application of Molecularly Imprinted Polymers for the Selective Extraction of Organophosphorus Pesticides from Vegetable Oils. *J. Chromatogr. A* **2017**, *1513*, 59–68. [[CrossRef](#)]
37. Varenne, F.; Kadhivel, P.; Bosman, P.; Renault, L.; Combès, A.; Pichon, V. Synthesis and Characterization of Molecularly Imprinted Polymers for the Selective Extraction of Oxazepam from Complex Environmental and Biological Samples. *Anal. Bioanal. Chem.* **2021**. [[CrossRef](#)]
38. Kadhivel, P.; Combès, A.; Bordron, L.; Pichon, V. Development and Application of Water-Compatible Molecularly Imprinted Polymers for the Selective Extraction of Carbamazepine from Environmental Waters. *Anal. Bioanal. Chem.* **2019**, *411*, 1525–1536. [[CrossRef](#)] [[PubMed](#)]
39. Pichon, V.; Haupt, K. Affinity Separations on Molecularly Imprinted Polymers with Special Emphasis on Solid-Phase Extraction. *J. Liq. Chromatogr. Relat. Technol.* **2006**, *29*, 989–1023. [[CrossRef](#)]