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1     **Molecularly imprinted sol-gel polymers for the analysis of iprodione fungicide**  
2                                     **in wine: synthesis in green solvent**

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9

10    **Abstract**

11    Iprodione is a fungicide widely used in viticulture in most agricultural countries. It was banned  
12    recently in the European community because of its carcinogenic and endocrine disrupting characters.  
13    In this work, a cheap analytical method able to monitor iprodione in a white wine was developed.  
14    Molecularly imprinted sol-gel polymers (MIS) specific to iprodione and using green solvents were  
15    synthesized. An experimental design having the following factors (solvent volume and crosslinker  
16    quantity) was used to prepare an optimal MIS. In terms of selectivity, the optimal MIS showed the  
17    best partition coefficient towards iprodione in a white wine containing four other competing  
18    fungicides (procymidone, pyrimethanil, azoxystrobin and iprovalicarb). A solid phase extraction  
19    method using the optimal MIS was optimized and applied to analyse iprodione in a white wine. Low  
20    detection and quantification limits were reached 11.7 and 39.1 µg/L respectively.

21

22    **Keywords**

23    Molecularly imprinted silica, fungicide, wine, experimental design, solid phase extraction, cheap  
24    method, green solvent

## 25 **1. Introduction**

26 Iprodione is a dicarboximide fungicide that is widely used in agriculture around the world. It was  
27 authorized to be used on vegetable crops, seed crops, fruit-tree crops, vines, ornamental crops,  
28 grasses, fragrance, food, medicinal and culinary plants and field crops (ANSES, 2017).

29 According to a pesticide-monitoring programme performed by the French Ministry of Agriculture  
30 (Cugier & Bruchet, 2005), iprodione was detected in 90% of the grapes analysed samples (47/52) and  
31 in 100% of the wines analysed samples (43/43).

32 In 2008, iprodione was classified as carcinogen category 2 in accordance with (EC) regulation  
33 N 1272/2008 of the European Parliament and of the council (Regulation EC 1272, 2008). Based on a  
34 European Food Safety Authority study, the European Community concluded that there is a high risk  
35 that the iprodione uses may expose groundwater to levels exceeding the drinking water limit of  
36 0.1 µg/L (European Food Safety Authority (EFSA), 2016). An evaluation conducted by the French  
37 Agency for Food, Environmental and Occupational Health and Safety (ANSES) in 2016 showed that  
38 iprodione has endocrine disruptor properties. For all these reasons, the (EU) regulation N 2017/2091  
39 decided not to renew the approval of iprodione. Any grace period accorded by the state members  
40 expired on 5 June 2018 (Regulation EU 2091, 2017).

41 In this context, a reliable and cheap method of iprodione analysis should be used in routine in order  
42 to check that agriculture products comply with this new regulation. Currently, chromatographic  
43 methods coupled to mass spectrometry are used to analysis fungicides in food and environmental  
44 samples (Castro et al., 2018; Elbashir & Aboul-Enein, 2018; Oliva et al., 2018). The big advantage of  
45 these methods is their low detection limits. However, they are expensive and require high technical  
46 experience for the lab staff. As an alternative, we proposed in a previous study a molecularly  
47 imprinted solid phase extraction method coupled to high performance liquid chromatography with a  
48 UV detection in order to analysis iprodione in wine (Bitar, Cayot, & Bou-Maroun, 2014). In this  
49 previous work, an acrylate base molecularly imprinted polymer (MIP) was used.

50 Molecularly Imprinted Polymers are synthetic materials widely used in analytical chemistry as  
51 preconcentration phase. They are shape memory materials. They act as a host for a guest target  
52 thanks to a double chemical and steric complementarity. They are able to recognize the target in a  
53 complex food (H. Li et al., 2018), **environmental matrices (Bakkour, Bolotin, Sellergren, & Hofstetter,**  
54 **2018; Ruggieri et al., 2015) or biological samples (Gao et al., 2017; Wei, Mu, Huang, & Liu, 2017).**

55 Molecularly imprinted polymers are synthesized in the presence of a template. After solubilization  
56 and interaction of a template molecule with a functional monomer in a solvent, a crosslinker is added  
57 to form the three-dimensional structure of the polymer in the presence of an initiator. After washing  
58 the polymer by an appropriate solvent, the template is removed leaving cavities highly specific to the  
59 target.

60 Most of the imprinted polymers studied in the literature are acrylate based polymers thanks to the  
61 high availability of commercial functional monomers and the ease of synthesis in organic solvents. In  
62 this work, sol-gel molecularly imprinted polymers (MIS) are developed instead of acrylate based  
63 molecularly imprinted polymer (MIP). As they are synthesized in a water/ethanol mixture, MIS  
64 respect one of the principle of green chemistry by using safer solvents than those used in MIP  
65 synthesis. Prat et al. studied solvents toxicity and elaborated a solvent classification from least toxic  
66 to most toxic. Water and ethanol are among the 9 recommended solvents of the 51 studied (Prat,  
67 Hayler, & Wells, 2014). Moreover, sol-gel MIS are more stable and specific towards the target species  
68 than acrylate based MIP, also allowing faster diffusion of analytes (da Costa Silva & Augusto, 2006).

69 **Molecularly imprinted polymers were already used as solid phase extraction (SPE) material in order**  
70 **to preconcentrate pesticides from real samples. Li et al. used MIS-SPE to extract carbendazim**  
71 **fungicide from vegetables before its determination by UPLC-UV with a detection limit of 3 ng/L (S. Li,**  
72 **Wu, Zhang, & Li, 2016). They used MIP-SPE to preconcentrate kelthane and pyridaben pesticides**  
73 **from vegetables before they analysis by GC-MS with a detection limit of 1 and 3.2 pg/g respectively**  
74 **(S. Li, Xu, Wu, & Luo, 2016).**

75 The first objective of this study was to obtain a sol-gel molecularly imprinted polymer (MIS) specific  
76 to iprodione. In order to get an optimal MIS able to pre-concentrate iprodione, a 2-factors  
77 experimental design was developed. In the MIS synthesis, the choice of the relative proportions of  
78 the reagents is a crucial point. For this reason, the following 2-factors were studied: the solvent  
79 volume and the crosslinker quantity. Selectivity was then checked using the optimal MIS in a white  
80 wine containing iprodione and four other competing fungicides. A solid phase extraction (SPE)  
81 method was developed using the optimal MIS to monitor iprodione in a white wine. The detection  
82 and the quantification limits of this method were determined and compared to the previous method  
83 based on MIP (Bitar et al., 2014) and to a SPE-LC-TOFMS method (Gilbert-López, García-Reyes,  
84 Mezcuca, Molina-Díaz, & Fernández-Alba, 2007).

85

## 86 **2. Material and methods**

### 87 *2.1. Chemicals*

88 Iprodione (97%, CAS number 36734-19-7), Azoxystrobin (Analytical standard, CAS number 131860-  
89 33-8), Iprovalicarb (Analytical standard, CAS number 140923-17-7), (3-Aminopropyl)trimethoxysilane  
90 (APTMS 97%, CAS number 13822-56-5), tetraethoxysilane (TEOS  $\geq 99\%$ , CAS number 78-10-4),  
91 ammonium hydroxide (NH<sub>4</sub>OH 28-30%, CAS number 1336-21-6), ethanol ( $\geq 99.8\%$ , CAS number 64-  
92 17-5), methanol (gradient grade, CAS number 67-56-1) and acetonitrile ( $\geq 99.9\%$ , CAS number 75-05-  
93 8) were purchased from Sigma Aldrich, France. Procymidone ( $\geq 98\%$ , CAS number 32809-16-8) and  
94 pyrimethanil ( $\geq 98\%$ , CAS number 53112-28-0) were bought from Chemos GmbH, Regenstauf,  
95 Germany. Water used in all experiments was deionised and obtained from an Elga Ionic system  
96 PURELAB Option.

97

### 98 *2.2. Synthesis of the molecularly imprinted and non-imprinted sol-gel polymers*

99 The molecularly imprinted sol-gel polymers (MIS) were prepared at 40 °C in a thermostatic water  
100 bath under magnetic stirring. The template molecule, iprodione was first solubilized in ethanol  
101 (iprodione is sparingly soluble in water, 16 mg·L<sup>-1</sup> for pH≤8, and slightly soluble in ethanol, 25 g·L<sup>-1</sup>;  
102 logP = 3.1; pKa = 9.2, logD = 2.25 at pH=10). Then, water was added, followed by the monomer  
103 APTMS (highly soluble in water, 1000 g·L<sup>-1</sup>, and ethanol, 100 g·L<sup>-1</sup>; pKa=10.6; from pH1 to 6, logD=-2;  
104 logD=0.37 at pH10) and the crosslinker TEOS (TEOS is sparingly soluble in water, 0.4 g·L<sup>-1</sup>, but reacts  
105 with water and slowly hydrolyses; TEOS is miscible with ethanol; logP=3.81). Finally, NH<sub>4</sub>OH was  
106 introduced. The reaction mixture was left under stirring for 20 hours. The polymers were separated  
107 from the liquid phase by centrifugation at 10 000 g for 10 minutes at room temperature. In order to  
108 eliminate iprodione and free the specific cavities, the polymers were washed several times with  
109 ethanol until iprodione was no longer detectable by reversed phase high performance liquid  
110 chromatography (HPLC) in washing solvents. After washing, the polymers were dried for 6 h at 60 °C.  
111 In parallel, NIS (Non Imprinted Silica) were synthesized under the same synthesis conditions as those  
112 of MIS, but without using the template molecule. NIS served as control polymers.

113 In a typical synthesis, the following molar ratios were used. For 7.5 or 15 mL of solvent, 0.1 mmol of  
114 iprodione, 0.4 mmol of APTMS, 2 or 6 mmol of TEOS and 10 mmol of NH<sub>4</sub>OH. The proportion  
115 ethanol/water was 90/10, v/v. In these conditions, the pH synthesis medium varies between 11.5  
116 and 11.7. In the sol-gel process, the polymer formation pass through hydrolysis of alkoxy silane than  
117 condensation. At low pH, the hydrolysis kinetic is fast and the condensation kinetic is slow. The  
118 condensation starts when hydrolysis is completed. At high pH, condensation is faster than hydrolysis.  
119 pH 11.5-11.7 was chosen to have a good compromise between the rate of hydrolysis and  
120 condensation.

121 In **figure 1**, the different chemical reactions are presented at the different steps, at the MIS synthesis  
122 step (A), washing step of the MIS (B) and and the mixing step with MIS and wine contaminated with  
123 iprodione where the interactions between the MIS and iprodione are involved (C).

124

125 *2.3. Binding properties and imprinting factor of MIS/NIS towards iprodione*

126 10 mg of imprinted polymer (MIS) or non-imprinted polymer (NIS) were suspended in 20 mL (50/50,  
127 v/v, ethanol/water) solutions containing 1 mmol·L<sup>-1</sup> of iprodione. The mixture was stirred for 1 h at  
128 25 °C. After removal of the polymer particles by centrifugation at 10 000 g for 10 min at room  
129 temperature, the supernatant was diluted 10 times, then filtered using a 2 µm filter and analysed by  
130 HPLC for the determination of iprodione.

131 For HPLC iprodione analysis, a Shimadzu HPLC (LC-20AT) pump equipped with a UV-Visible detector  
132 (SPD-20A), a Restek column (Pinacle II, C18 5 µm, 150 x 4.6 mm) and a pre-column (C18, 5 µm, 10 x  
133 4 mm) were used. Separations were conducted at room temperature. Isocratic acetonitrile/water  
134 (60/40, v/v) was used as mobile phase and the flow rate was 1mL/min. Iprodione detection was done  
135 at 220 nm. A calibration curve was constructed over the range 0.1–500 µmol·L<sup>-1</sup> of iprodione. **Eight**  
136 **standards were prepared in (50/50, v/v, ethanol/water) solutions, the calibration curve equation was**  
137  **$y = 47820 x + 254129$  ( $y = \text{peak area}$ ;  $x = \text{concentration}$ ) and the correlation coefficient  $R^2 = 0.9989$ .**

138 The amount of iprodione retained by the polymer (B, mg of iprodione/g of polymer) was calculated  
139 by difference between the initial iprodione concentration (C<sub>0</sub>, mmol/L) and the free iprodione  
140 concentration (F, mmol/L) in the supernatant.

141 The imprinting factor (IF) was calculated by the equation (Spivak, 2005):

142 
$$IF = \frac{K(MIS)}{K(NIS)} = \frac{B(MIS) \times F(NIS)}{F(MIS) \times B(NIS)}$$

143

144 where K is the partition coefficient  $K = B/F$ . The imprinting factor evaluates the specific binding  
145 property of each polymer towards iprodione. It takes into account the sorption of iprodione by a MIS

146 and its corresponding control polymer NIS. It allows to get rid of the non-specific interactions that  
147 result from the iprodione adsorption on NIS.

148 Statistical analysis (ANOVA with Tukey's post hoc analysis) was done using (XLSTAT, Addinsoft)  
149 software.

150

#### 151 *2.4. Selectivity studies*

152 20 mg of MIS or NIS were suspended in 10 mL of a Burgundy white wine (Chardonnay, Charles  
153 Renoir). The same wine was supplemented by the following fungicides: iprodione, procymidone,  
154 pyrimethanil, azoxystrobin and iprovalicarb at 0.2 mmol·L<sup>-1</sup> of each fungicide (cf. figure S1 of the  
155 supplementary material section for the fungicide chemical structures). 500 µL of the MIS or NIS  
156 suspension was mixed with 500 µL of the supplemented wine in a 2 mL Eppendorf tube. The tubes  
157 were then agitated for 3 h using a Stuart rotator SB2. The tubes were finally centrifuged for 10 min at  
158 20 000 *g* at room temperature. The supernatant were analysed by HPLC using the same system  
159 described in the paragraph 2.3 in order to determine the concentration of each of the studied  
160 fungicides. Each experiment was repeated three times.

161 The following gradient was used to separate the 5 fungicides: (solvent A water, solvent B acetonitrile)  
162 50% of B from 0 to 2 min, 70% of B from 2 to 8 min, 90% of B from 8 to 10 min, 50% of B from 10 to  
163 12 min. Finally, a 2 min reconditioning step was applied at the initial conditions. A calibration curve  
164 was constructed over the range 0.1–500 µmol·L<sup>-1</sup> of each fungicide. Eight standards were prepared in  
165 (90/10, v/v, water/ethanol) solutions. The calibration curve equations and the correlation  
166 coefficients were  $y = 18648x + 18401$  ( $R^2 = 0.9991$ ) for iprodione;  $y = 10682x + 8412$  ( $R^2 = 0.9993$ ) for  
167 procymidone;  $y = 7428x - 2736$  ( $R^2 = 0.9996$ ) for pyrimethanil;  $y = 30934x + 7228$  ( $R^2 = 0.9994$ ) for  
168 azoxystrobin and  $y = 7217x - 3109$  ( $R^2 = 0.9996$ ) for iprovalicarb.

169 Statistical analysis (ANOVA with Tukey's post hoc analysis) was done using (XLSTAT, Addinsoft)  
170 software.

171

## 172 *2.5. Molecularly Imprinted Solid Phase Extraction (MISPE)*

173 Empty polypropylene SPE cartridge (1 mL, Supelco, Bellefonte, USA) was packed with 20 mg of MIS or  
174 NIS previously suspended in acetonitrile. The polymers (particle size > 20  $\mu\text{m}$ ) were packed between  
175 two polyethylene frits of 20  $\mu\text{m}$  porosity at each end. The SPE cartridges were firstly preconditioned  
176 with 5 mL of methanol or ethanol and then with 5 mL of (water/ethanol, v/v, 87/13). 6 mL white  
177 wine sample (Chardonnay, Charles Renoir) spiked with ( $5 \mu\text{mol}\cdot\text{L}^{-1} = 1.65 \mu\text{g}/\text{mL}$ ) of iprodione was  
178 loaded. The cartridges were subsequently washed with 2 mL of washing solvents, mixture of  
179 water/acetone at different proportions: 70/30, 50/50 or 25/75, v/v. The elution step was performed  
180 using 1 mL of elution solvents: water/acetone, where the acetone proportion was varied between 75  
181 and 100% (v/v). The effluents from the different SPE steps were analysed by HPLC in order to  
182 quantify iprodione using the same conditions described in paragraph 2.3. A calibration curve was  
183 constructed over the range  $0.1\text{--}5 \mu\text{mol}\cdot\text{L}^{-1}$  of iprodione. All experiments were done in triplicate.

184

## 185 *2.6. Characterisation of the optimal sol-gel polymer*

186 Fourier transform infrared (FTIR) spectra were recorded on a Perkin Elmer spectrum 65 FT-IR  
187 spectrometer in the range  $4000\text{--}500 \text{ cm}^{-1}$  using attenuated total reflectance sampling. 64 scans with  
188 a resolution of  $4 \text{ cm}^{-1}$  were applied.

189 For the surface morphological characterization and Energy-dispersive X-ray (EDX) spectrum, samples  
190 were suspended in ethanol then a drop was placed on a silicon grid and examined in Hitachi Scanning  
191 Electron Microscope (SEM) SU1510.

192 The size distribution of the optimal polymer was measured by laser diffraction (Malvern Mastersizer  
193 3000) after suspending 20 mg of polymer powder in 10 mL of water and sonication for 2 min.

194

### 195 3. Results and discussion

#### 196 3.1. Experimental design for the determination of the optimal polymer

197 The objective of the experimental design was to develop an optimal polymer for the  
198 preconcentration of iprodione fungicide from wine samples. The optimal polymer must have the  
199 highest adsorption capacity and the best imprinting factor toward iprodione. The following 2<sup>2</sup>  
200 experimental design was applied (**table 1**).

201 **Table 1. Coded levels and corresponding factors of the 2<sup>2</sup> experimental design.**

Factors	X <sub>1</sub>	X <sub>2</sub>
	Solvent volume (V, mL)	Crosslinker quantity (Q, mmol)
Levels		
-1	7.5	2
1	15	6

202

203 The solvent volume (X<sub>1</sub> = V) and the crosslinker quantity (X<sub>2</sub> = Q) and were selected as the two factors  
204 of the experimental design. Two levels coded (-1) and (+1) were considered for each factor. This  
205 experimental design implied the synthesis of 4 MIS (MISa, MISb, MISc and MISd). In parallel to the  
206 MIS preparation, 4 NIS were synthesized. They constituted control polymers for the corresponding  
207 MIS.

208 The influence of X<sub>1</sub> and X<sub>2</sub> factors on the following two variables: the imprinting factor (Y<sub>1</sub> = IF) and  
209 the iprodione adsorbed amount (Y<sub>2</sub> = B) was studied. Y<sub>1</sub> and Y<sub>2</sub> represent the responses of the  
210 experimental design . The determination of IF and B was done three times for each polymer. The  
211 results are compiled in **table 2**.

212

213 **Table 2. Experimental design factors (X) and responses (Y). Q (crosslinker quantity), S (solvent volume), IF**  
 214 **(Imprinting Factor), B (adsorbed iprodione, mg of iprodione/g of polymer).**

Experiment N°	Polymer	X <sub>1</sub> = V	X <sub>2</sub> = Q	Y <sub>1</sub> = IF	Y <sub>2</sub> = B (mg/g)
1	MISa,1	-1	-1	3.07	15.03
2	MISa,2	-1	-1	3.37	13.74
3	MISa,3	-1	-1	3.22	14.38
4	MISb,1	-1	1	0.69	27.34
5	MISb,2	-1	1	0.84	26.85
6	MISb,3	-1	1	0.88	27.95
7	MISc,1	1	-1	1.58	12.49
8	MISc,2	1	-1	1.69	13.38
9	MIS,c3	1	-1	1.69	11.05
10	MISd,1	1	1	0.84	12.61
11	MISd,2	1	1	0.79	11.94
12	MISd,3	1	1	0.81	12.27

215

216 For each response, the calculated model from the experimental design is expressed as follows:

217  $Y (\text{response}) = b_0 + b_1 \cdot X_1 + b_2 \cdot X_2 + b_{12} \cdot X_1 X_2$

218  $b_0$  is the response average.  $b_1$  and  $b_2$  are coefficient of the respective factors  $X_1$  and  $X_2$ , they  
 219 represent factors effect on Y. The equation resolution for the 2 responses  $Y_1$  and  $Y_2$  gave:

220  $Y_1 = 1.6253 - 0.3912 X_1 - 0.8154 X_2 + 1.5699 X_1 X_2$

221  $Y_2 = 16.5901 - 4.2962 X_1 + 3.2405 X_2 - 13.0241 X_1 X_2$

222 The significance limit for each response was calculated based on the standard deviation of all  
 223 experiments "S", it was equal to 1.96S. The significance limit was respectively 0.188 and 1.620 for  $Y_1$   
 224 and  $Y_2$ . For both responses, the interactions between the two factors (solvent volume and crosslinker  
 225 quantity) were the most significant. **Results of ANOVA and regression applied to the response of the**  
 226 **design of experiment are presented in tables S1 and S2 of the supplementary material section.**

227 **Figure 2** shows that as the solvent volume (V) increases during the synthesis step, the imprinting  
 228 factor decreases or remain constant (fig.2A) and the quantity of adsorbed iprodione decreases  
 229 (fig.2B). This observation could be explained by a dilution effect. The higher the solvent volume, the

230 lower the reactants meeting probability with monomers, the fewer the cavities specific to iprodione.  
231 Therefore, there is a great interest in decreasing the solvent volume during the MIS synthesis.

232 Concerning the quantity of crosslinker (Q): When Q increases, the imprinting factor decreases (fig.2A)  
233 but the iprodione binding property remain constant or increases (fig.2B). This observation could be  
234 explained by the increasing of the non-specific interactions between the iprodione and the polymers.  
235 In this case, the non-specific interactions are responsible for the amelioration of iprodione binding  
236 properties. Therefore, there is no interest in increasing the crosslinker quantity during the MIP  
237 synthesis.

238 **Table 2** shows that MISb (V = 7.5 mL and Q = 6 mmol) had the best iprodione binding property  
239 (27.4 mg/g). However, it had the smallest imprinting factor (0.81). MISa (V = 7.5 mL and Q = 2 mmol)  
240 had the best imprinting factor (3.2) and the second best iprodione binding property (14.4). Based on  
241 the experimental design results, MISa was chosen as the optimal MIS.

242 The optimal MIS imprinting factor was 3.2. It is higher than the one we determined in a previous  
243 study (IF = 2.4) (Bitar et al., 2015) that aimed to develop acrylate-based MIP specific to iprodione. On  
244 the other hand, the optimal MIS had an adsorption capacity of 14.4 mg/g (mg of iprodione per g of  
245 MIS). It is 33 times lower than that of the MIP, which was equal to 473 mg/g. This comparison of the  
246 acrylate-based (MIP) and the silica-based (MIS) polymers shows that the MIS are more selective but  
247 have a relatively low adsorption capacity which is in agreement with the following studies reports in  
248 literature (Cummins, Duggan, & McLoughlin, 2005; Marx & Liron, 2001).

249

### 250 *3.2. Characterization of the optimal MISa*

251 The optimal MISa was characterized by Scanning Electron Microscopy, laser diffraction  
252 particle size analyser and Fourier transform infrared (FTIR) spectroscopy. Results are  
253 presented in supplementary materials (figures S2 to S5). SEM micrographs show that the

254 bigger particles size of MISa range from 18 to 61  $\mu\text{m}$ . Particle size distribution of MISa show  
255 that the average particle size is  $13 \pm 3 \mu\text{m}$ . The EDX spectrum show 3 major peaks  
256 corresponding to oxygen (52.6%), silicon (29.9%) and carbon (16.6%). These percentages  
257 show that (3-Aminopropyl)trimethoxysilane (APTMS) monomer reacts with  
258 tetraethoxysilane (TEOS) to form the sol-gel polymer. FTIR spectrum of MISa show that all  
259 the observed peaks are related to the absorption of the TEOS crosslinker and the APTMS  
260 monomer after the sol-gel hydrolysis and condensation. The condensed APTMS have several  
261 absorption bands: the ones at  $2984 \text{ cm}^{-1}$  and  $790 \text{ cm}^{-1}$  correspond to the C-H group and the  
262 one at  $960 \text{ cm}^{-1}$  corresponds to the C-C group. The condensed TEOS has an absorption band  
263 at  $1060 \text{ cm}^{-1}$  corresponding to the Si-O-Si group.

264

### 265 *3.3. Selectivity of the optimal polymer MISa*

266 The selectivity of the optimal polymer MISa was studied in a Burgundy white wine supplemented  
267 with the five following competing fungicides: iprodione, procymidone, pyrimethanil, iprovalicarb and  
268 azoxystrobin. The fungicides choice was based on a study done by the French Ministry of Agriculture  
269 in 13 regions representing the main French vineyards. The chosen fungicides were detected in most  
270 of the 1 222 wine analysed samples (Cugier & Bruchet, 2005).

271 The partition coefficient K (mL/g) was calculated for each fungicide. The fungicides partition was  
272 determined between MISa or NISa and the white wine. K values were calculated using the equation  
273 (Duan, Yi, Fang, Fan, & Wang, 2013):

$$274 \quad K = \frac{B}{F} \times \frac{V}{m}$$

275 where B is the fungicide amount retained by the polymer and F the free amount remaining in wine.  
276 V (mL) is the wine volume and m (g) is the polymer mass. The obtained results are presented in  
277 **figure 3.**

278 Iprodione had the highest K MIS value followed by procymidone. These values are coherent because  
279 MISa was synthesized using iprodione as template. Procymidone has a close chemical structure to  
280 iprodione compared to the other fungicides. The same order was maintained for K NIS values.  
281 However, K NIS values were much lower than K MIS values. This shows that the molecular imprinting  
282 process was successful.

283 The selectivity of MISa was assessed by the selectivity coefficient  $\alpha$  defined as follows:

$$284 \quad \alpha = \frac{K_{MIS}(\text{fungicide})}{K_{MIS}(\text{template})}$$

285 Where the template is iprodione. The calculated  $\alpha$  values are: 1.1, 1.2, 1.7 and 2.3. They correspond  
286 respectively to procymidone, azoxystrobin, pyrimethanil and iprovalicarb. Procymidone, the  
287 fungicide having the closest chemical structure to iprodione, had the lowest  $\alpha$  value. Iprovalicarb,  
288 with the most distant chemical structure from iprodione, had the highest  $\alpha$  value.

289 The optimal MIS was able to interact with iprodione but to a lesser extent with other competing  
290 fungicides in a white wine sample. In order to eliminate the non-specific interactions and to pre-  
291 concentrate only iprodione, an optimisation of molecularly imprinted solid phase extraction was  
292 performed.

293

#### 294 **3.4. Determination of iprodione in white wine by Molecularly Imprinted Solid Phase**

##### 295 *Extraction (MISPE) using the optimal MISa*

296 Before the application of MISa in the preconcentration of iprodione from a white wine using solid  
297 phase extraction (SPE), the optimization of the solvents used during the different steps of the SPE

298 was done. MISa and NISa were packed into two different cartridges. The SPE cartridge filled with  
 299 MISa was washed with 10 mL of acetonitrile in order to eliminate any residual iprodione. Methanol  
 300 and ethanol was tested as pre-conditioning solvent. Methanol allowed better sorption of iprodione  
 301 and gave a better sorption difference between MISa and NISa. The conditioning solvent was  
 302 water/ethanol, 90/10, v/v. After loading the white wine supplemented with iprodione, three washing  
 303 solvents were tested: water/acetone, v/v, 70/30, 50/50 and 25/75 respectively. The latter were  
 304 chosen because it allowed the elution of the total amount of iprodione adsorbed on the NIS. Several  
 305 elution solvents were used in order to elute all iprodione adsorbed on the MIS. For this reason, the  
 306 acetone proportion was varied between 75% and 100%. Acetone 100% eliminated the total amount  
 307 of iprodione adsorbed by MISa. The adsorbed and recovered amount of iprodione during the  
 308 different SPE steps are compiled in **table 3**.

309 **Table 3. Adsorbed and recovered amount of iprodione from white wine using MISPE. Means  $\pm$  SD (n = 3).**

Polymer	Adsorbed ( $\mu\text{g}$ )	Washing recovery ( $\mu\text{g}$ )	Elution recovery ( $\mu\text{g}$ )	Total recovery (%)
MISa	8.55 $\pm$ 0.85	2.95 $\pm$ 0.21	4.43 $\pm$ 0.82	86 $\pm$ 10
NISa	3.71 $\pm$ 0.73	3.11 $\pm$ 0.41	0	84 $\pm$ 9

310

311 MISa adsorbed twice as much iprodione as NISa. The washing step removed all of the non-specific  
 312 interactions that correspond to the amount of iprodione adsorbed on NISa. The iprodione recovered  
 313 by the elution step corresponds to the specific interactions. It represents 52% of the adsorbed  
 314 iprodione. Acceptable total recovery percentages (higher than 80 %) were obtained for both MISa  
 315 and NISa.

316 Blank white wine was analyzed by HPLC in order to check the specificity of the developed method.  
 317 The blank chromatograms showed no interfering peaks at the retention time of iprodione. The limit  
 318 of detection (LOD) and the limit of quantification (LOQ) of the MISPE method were calculated using

319 the following equations:  $LOD = (3.3/6) (SD/S)$  ;  $LOQ = (10/6) (SD/S)$  where SD is the standard  
320 deviation of the HPLC response and S is the slope of the calibration curve. 6 corresponds to the  
321 preconcentration factor of the MIS-SPE method. The calculated LOD and LOQ were respectively 11.7  
322 and  $39.1 \mu\text{g}\cdot\text{L}^{-1}$ . The relative standard deviation of the method ( $n = 3$ ) was 10 %. The quantification  
323 limit of this method is lower than that determined by MIP-SPE-HPLC in our previous studies in white  
324 wine ( $LOQ = 422 \mu\text{g}\cdot\text{L}^{-1}$ ) (Bitar et al., 2014). It is comparable to that determined by SPE-LC-TOFMS in  
325 fruit juices ( $LOQ = 1.5 \mu\text{g}\cdot\text{L}^{-1}$ ) (Gilbert-López et al., 2007) but really less expensive.

326 Currently, the fungicides detection methods used are mainly based on mass spectrometric detection  
327 and are relatively expensive (Gilbert-López et al., 2007; Hayward & Wong, 2009; Wong et al., 2010).  
328 In parallel, ELISA methods using an immunoabsorbent are used (Koch, Stier, Senseman, Sobek, &  
329 Kerns, 2013; Watanabe & Miyake, 2007). In addition to their high cost, these methods cannot be  
330 used in extreme pH or temperature environments. Preconcentration methods using molecularly  
331 imprinted polymers (MIPs) could replace the methods currently used. MIPs are inexpensive materials  
332 and resist to extreme temperature and pH conditions (Haupt, 2001; Svenson & Nicholls, 2001).  
333 The developed MIS-SPE-HPLC method has two major limitations: it is time consuming and cannot be  
334 applied for fungicides that do not absorb in the UV-visible range.

335

#### 336 4. Conclusion

337 Molecularly imprinted sol-gel silica (MIS) were developed in this work in order to conduct synthesis  
338 in safe condition, and to preconcentrate iprodione fungicide from a Burgundy white wine using solid  
339 phase extraction. The analysis was done by a cheap HPLC-UV method. To prepare the optimal MIS for  
340 iprodione, an experimental design having the following 2 factors (solvent volume and crosslinker  
341 quantity) was developed. The responses of the experimental design were the imprinting factor (IF)  
342 and the binding capacity (BC). The optimal MIS had a higher IF but a lower BC than a previously

343 synthesized acrylate based polymer (MIP) specific to iprodione. The design of experiment showed  
344 that the optimal MIS with the higher IF and BC, was obtained with low solvent volume and low  
345 crosslinker quantity during its synthesis. The selectivity of the optimal MIS was checked in a white  
346 wine containing iprodione and four other competing fungicides. The fungicide with the closest  
347 chemical structure to iprodione gave the lowest selectivity factor and the fungicide with the most  
348 distant chemical structure to iprodione gave the highest selectivity factor. The optimal MIS was used  
349 as packing material in a solid phase extraction (SPE) to pre-concentrate iprodione from a white wine.  
350 After the optimization of the different steps of the SPE, MISPE-HPLC-UV method was able to detect  
351 iprodione in a white wine with a detection limit of  $11.7 \mu\text{g}\cdot\text{L}^{-1}$ . This method could be used to monitor  
352 this forbidden fungicide in wine easily and with a cheap lab equipment such as an UV-VIS-HPLC.

353

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360

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467

468

469 **Figure captions:**

470 **Figure 1.** Proposed chemical reactions involved in the MIS synthesis. A. Basic hydrolysis and poly-  
471 condensation of tetraethoxysilane crosslinker and (3-Aminopropyl)trimethoxysilane functional  
472 monomer in the presence of iprodione (in blue). B. Removal of iprodione from the polymer by  
473 washing with ethanol. C. Rebinding of iprodione (in blue) in the specific cavities.

474

475 **Figure 2.** Imprinting factor  $\pm$  SD (n = 3) (A) and binding capacity  $\pm$  SD (n = 3) (B) of the MIPs towards  
476 iprodione in accordance with of the experimental design. Q is the crosslinker quantity used during  
477 the MIP synthesis. Statistical comparisons were done by ANOVA with Tukey's post hoc analysis.  
478 Values significantly different (P<0.05) are indicated by different small letters.

479

480 **Figure 3.** Fungicides partition coefficients between MISa or NISa and a Burgundy white wine spiked  
481 with iprodione, procymidone, azoxystrobin, pyrimethanil and iprovalicarb at 0.1  $\mu\text{mol/mL}$  of each  
482 fungicide. Values are means  $\pm$  SD (n = 3/group) partition coefficient. Statistical comparisons were  
483 done by ANOVA with Tukey's post hoc analysis. Values significantly different (P<0.05) between  
484 fungicides are indicated by different letters. K MIS and K NIS values were processed separately.

485

486

**Figure 1.**

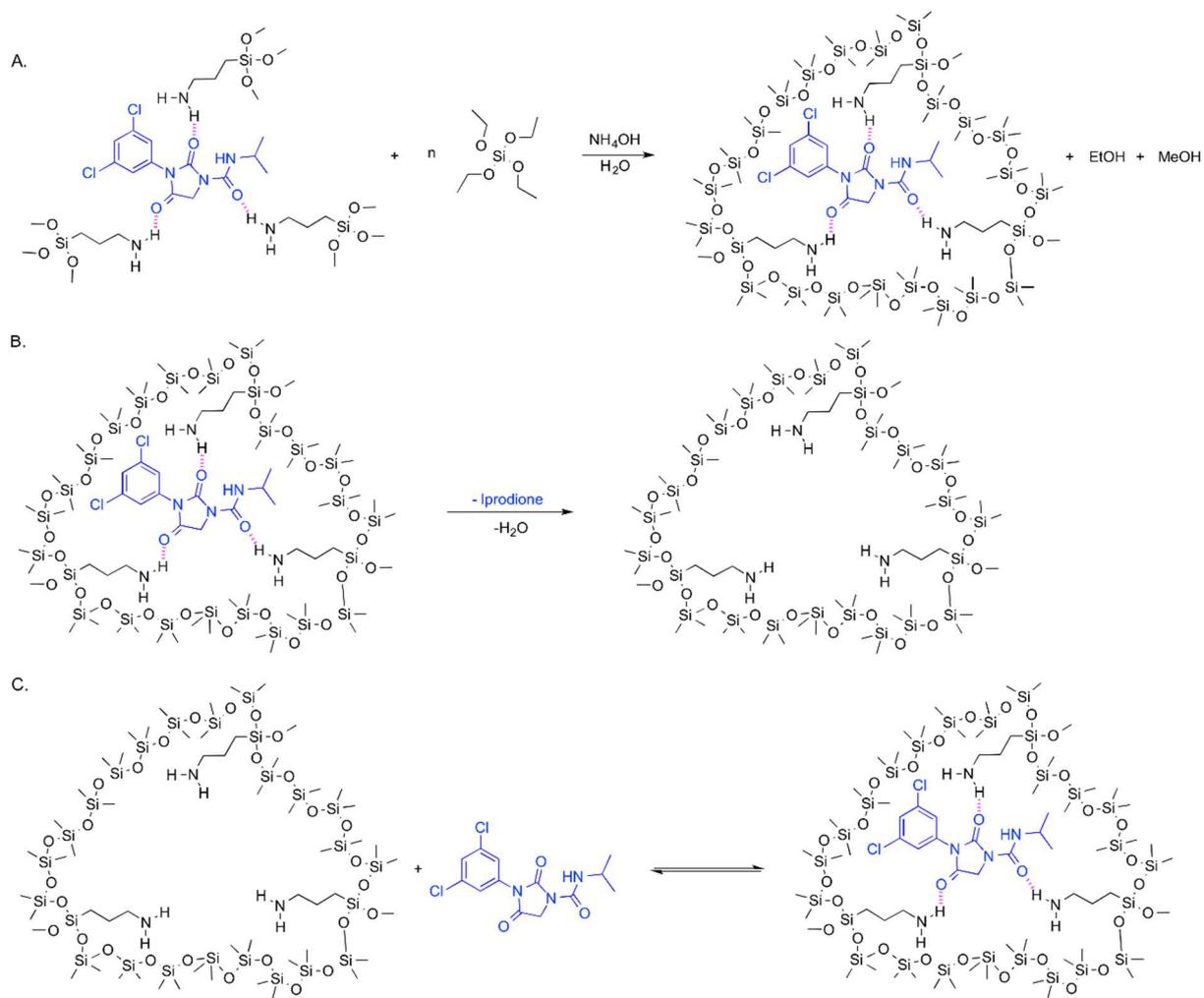


Figure 2.

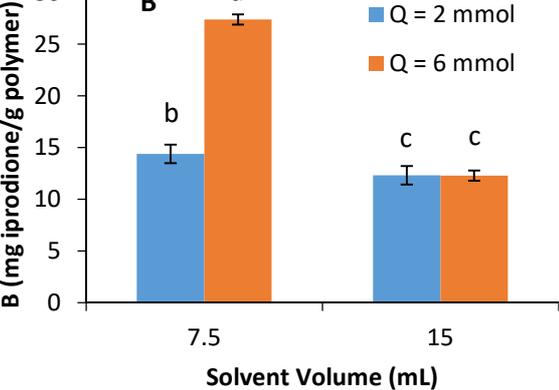
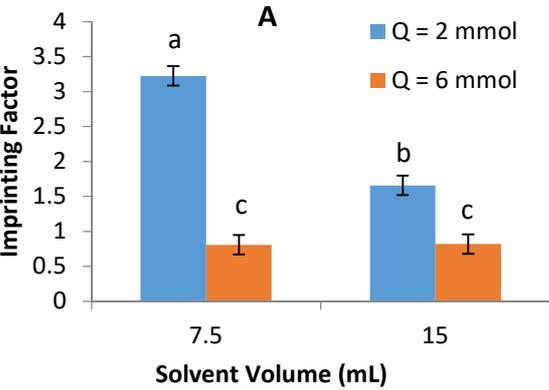


Figure 3.

