Molecularly imprinted sol-gel polymers for the analysis of iprodione fungicide in wine: Synthesis in green solvent

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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**

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1. Introduction

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

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

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

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

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

2. Material and methods

2.1. Chemicals

Iprodione (97%, CAS number 36734-19-7), Azoxystrobin (Analytical standard, CAS number 131860-33-8), Iprovalicarb (Analytical standard, CAS number 140923-17-7), (3-Aminopropyl)trimethoxysilane (APTMS 97%, CAS number 13822-56-5), tetraethoxysilane (TEOS ≥99%, CAS number 78-10-4), ammonium hydroxide (NH₄OH 28-30%, CAS number 1336-21-6), ethanol (≥99.8%, CAS number 64-17-5), methanol (gradient grade, CAS number 67-56-1) and acetonitrile (≥99.9%, CAS number 75-05-8) were purchased from Sigma Aldrich, France. Procymidone (≥98 %, CAS number 32809-16-8) and pyrimethanil (≥98 %, CAS number 53112-28-0) were bought from Chemos GmbH, Regenstauf, Germany. Water used in all experiments was deionised and obtained from an Elga Ionic system PURELAB Option.

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

4

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^{-1} for pH≤8, and slightly soluble in ethanol, 25 g·L^{-1};

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^{-1}, and ethanol, 100 g·L^{-1}; 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^{-1}, but reacts

105   with water and slowly hydrolyses; TEOS is miscible with ethanol; logP=3.81). Finally, NH_4OH 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_4OH. 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 alkoxysilane 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

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

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

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

The amount of iprodione retained by the polymer (B, mg of iprodione/g of polymer) was calculated by difference between the initial iprodione concentration (C_0, mmol/L) and the free iprodione concentration (F, mmol/L) in the supernatant.

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

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

where K is the partition coefficient K = B/F. The imprinting factor evaluates the specific binding 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^{-1} 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^{-1} 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 μm) were packed between

175 two polyethylene frits of 20 μm 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 μmol·L^{-1} = 1.65 μg/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 0.1–5 μmol·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 4000–500 cm^{-1} using attenuated total reflectance sampling. 64 scans with

188 a resolution of 4 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 2^2

200 experimental design was applied **(table 1)**.

201 **Table 1. Coded levels and corresponding factors of the 2^2 experimental design.**

Factors	X_1	X_2
	Solvent volume	Crosslinker quantity
Levels	(V, mL)	(Q, mmol)
-1	7.5	2
1	15	6

202

203 The solvent volume (X_1 = V) and the crosslinker quantity (X_2 = 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_1 and X_2 factors on the following two variables: the imprinting factor (Y_1 = IF) and

209 the iprodione adsorbed amount (Y_2 = B) was studied. Y_1 and Y_2 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	$X_1 = V$	$X_2 = Q$	$Y_1 = IF$	$Y_2 = B$ (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 (response) = $b_0 + b_1.X_1 + b_2.X_2 + b_{12}.X_1X_2$

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 $Y_1 = 1.6253 - 0.3912 X_1 - 0.8154 X_2 + 1.5699 X_1X_2$

221 $Y_2 = 16.5901 - 4.2962 X_1 + 3.2405 X_2 - 13.0241 X_1X_2$

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

bigger particles size of MISa range from 18 to 61 μm. Particle size distribution of MISa show

255 that the average particle size is 13 ± 3 μm. 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 cm^{-1} and 790 cm^{-1} correspond to the C-H group and the

262 one at 960 cm^{-1} corresponds to the C-C group. The condensed TEOS has an absorption band

263 at 1060 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
$$K = \frac{B}{F} \times \frac{V}{m}$$

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 α defined as follows:

284 $$\alpha = \frac{K\ MIS\ (fungicide)}{K\ MIS\ (template)}$$

285   Where the template is iprodione. The calculated α 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 α value. Iprovalicarb,

288   with the most distant chemical structure from iprodione, had the highest α 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 ± SD (n = 3).*

Polymer	Adsorbed (μg)	Washing recovery (μg)	Elution recovery (μg)	Total recovery (%)
MISa	8.55 ± 0.85	2.95 ± 0.21	4.43 ± 0.82	86 ± 10
NISa	3.71 ± 0.73	3.11 ± 0.41	0	84 ± 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 $\mu g \cdot L^{-1}$. 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 $\mu g \cdot L^{-1}$) (Bitar et al., 2014). It is comparable to that determined by SPE-LC-TOFMS in

325 fruit juices (LOQ = 1.5 $\mu g \cdot L^{-1}$) (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 immunoadsorbent 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 $\mu g \cdot L^{-1}$. 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

360

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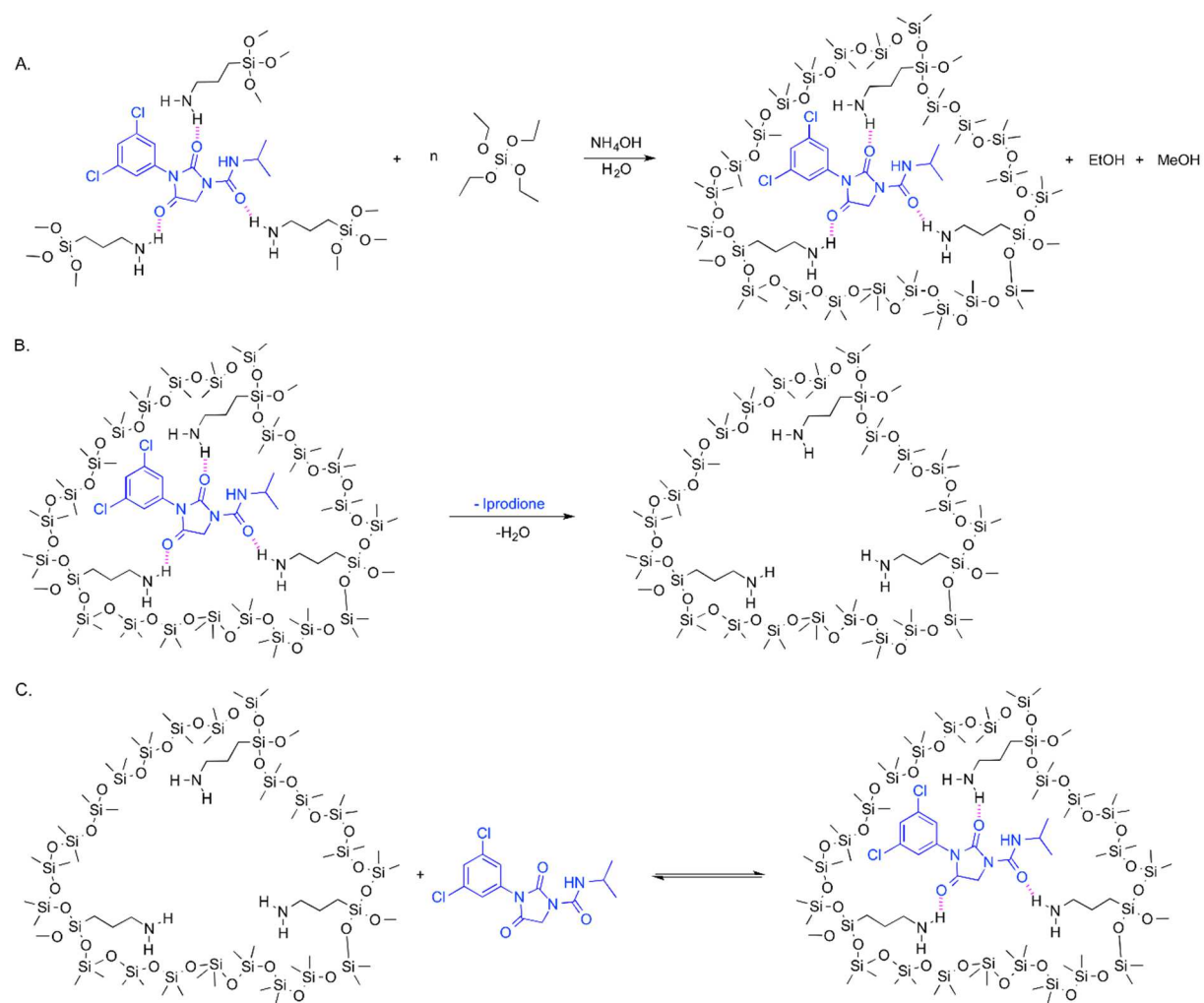465 MS/MS). *Journal of Agricultural and Food Chemistry*, *58*(10), 5884–5896.

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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 ± SD (n = 3) (A) and binding capacity ± 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.
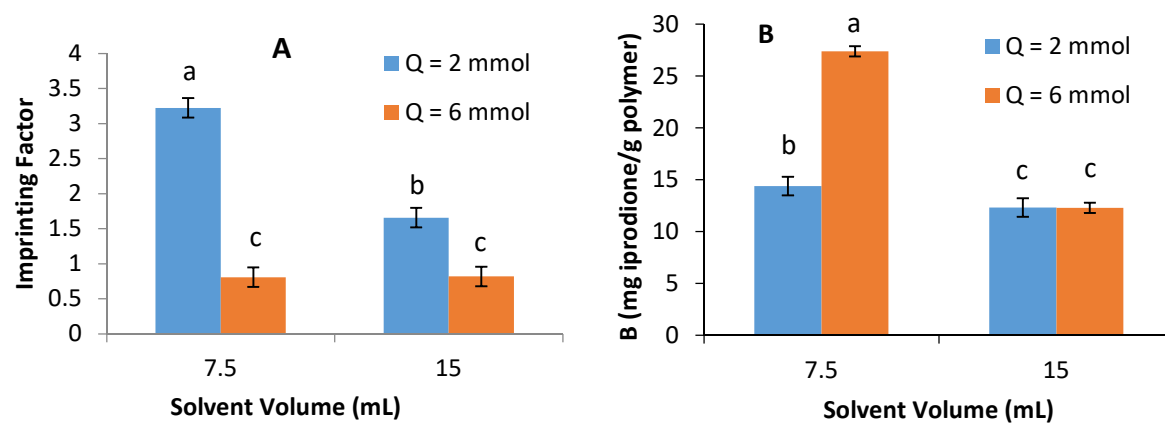
479

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 μmol/mL of each

482 fungicide. Values are means ± 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.