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Silica-supported carbon from Kraft lignin pyrolysis for solid-phase extraction of fluoroquinolone probes pollutants from environmental waters --Manuscript Draft--

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Abstract:	<p>This work presents a novel carbon-based material obtained from a low cost biomass, and its original application for solid-phase extraction (SPE) of organic pollutants from natural waters. Marbofloxacin (MAR) and Enrofloxacin (ENR), two widely used fluoroquinolones (FQs), were chosen as model emerging contaminants. The material was easily prepared by adsorption of lignin (LG) onto silica microparticles followed by pyrolytic treatment at 1200°C for 2 h. Silica-supported lignin (LG-silica) was characterized by scanning electron microscopy (SEM), surface area measurement (BET), thermogravimetric analysis (TGA) and electron paramagnetic resonance (EPR). LG-silica (200mg) was tested as column-packed SPE sorbent for pre-concentration of tap and river water spiked with MAR and ENR in the range 10-1000 ng L⁻¹ (n=3). Quantitative adsorption was observed at the sample native pH (~7.5) for both drugs, which were eluted from the cartridge with 2 x 2 mL aqueous tetrabutyl ammonium hydroxide-acetonitrile (70:30), and quantified by high performance liquid chromatography coupled to fluorescence detection. Recoveries in the range 70-116% and suitable inter-day precision (RSDs 7-16%) were observed for 20-1000 ng L⁻¹ spikes, also in raw river water. Sample volumes up to 500 mL provided enrichment factors up to 125, useful for determination at environmentally significant levels (few ng L⁻¹) and, at the same time, attractive compared to the commercial sorbents. The batch-to-batch reproducibility was verified on 3 LG-silica preparations. The novel sorbent phase was successfully applied to the determination of these drugs in actual environmental waters.</p>

1 **Silica-supported carbon from Kraft lignin pyrolysis for solid-phase extraction of**
2 **fluoroquinolone probes pollutants from environmental waters**

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30 **Abstract**

31 This work presents a novel carbon-based material obtained from a low cost biomass, and its original application for
32 solid-phase extraction (SPE) of organic pollutants from natural waters. Marbofloxacin (MAR) and Enrofloxacin (ENR),
33 two widely used fluoroquinolones (FQs), were chosen as model emerging contaminants. The material was easily
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35 Silica-supported lignin (LG-silica) was characterized by scanning electron microscopy (SEM), surface area
36 measurement (BET), thermogravimetric analysis (TGA) and electron paramagnetic resonance (EPR). LG-silica
37 (200mg) was tested as column-packed SPE sorbent for pre-concentration of tap and river water spiked with MAR and
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42 river water. Sample volumes up to 500 mL provided enrichment factors up to 125, useful for determination at
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44 The batch-to-batch reproducibility was verified on 3 LG-silica preparations. The novel sorbent phase was successfully
45 applied to the determination of these drugs in actual environmental waters.

46
47 **Keywords:** Emerging pollutants; Fluoroquinolones; Lignin; Pyrolysis; Solid-phase extraction; Surface water

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60 **Introduction**

61 Reversed-phase materials have been commonly used in separation science for pre-concentration of a variety of organic
62 compounds from aqueous solution [1]. Beside the conventional C18-silica, in the recent years novel materials have been
63 prepared and applied for analytical purposes, exploiting the exceptional properties of new forms of sp² carbon, such as
64 carbon nanotubes and more recently graphene [2-4]. The latter, made of single-layer or few-layer thickness of sp²-
65 hybridized carbon atoms arranged in a honeycomb-like structure, showed great potentiality to prepare efficient sorbents
66 for solid-phase extraction (SPE) of pollutants [5, 6]. Differently from C18-silica, an aspecific sorbent merely based on
67 hydrophobic effects, graphene exerts several interplays with target species, *viz.* π - π stacking, cation- π bonding, electron
68 donor-acceptor and hydrophobic interactions, hydrogen bond, etc. [5]. Comparable or even better performance was
69 observed in comparison with carbon nanotubes, graphitic carbon, and commercial sorbents for pre-concentration of
70 various contaminants, e.g. sulfonamides [7] and fluoroquinolones (FQs) [8]. These, antibiotics for human and veterinary
71 medicine endowed with broad activity spectrum and good oral absorption, are important emerging contaminants. In the
72 recent years their occurrence has been assessed both in aquatic [9] and soil [10] compartments at concentrations in the
73 ranges ng- μ g L⁻¹ and μ g-mg kg⁻¹, respectively. Various reasons stand for the environmental widespread, primarily the
74 partial metabolization and not quantitative abatement in wastewater treatment plants (WWTPs) [11]. This involves the
75 release of variable quantities of pharmaceutically active drugs into water basins, affecting transformation/removal rates.
76 The common practice of recycling manure from livestock farming and sewage sludge from WWTPs as fertilizers, and
77 recycling of sludge for the production of compost, widely employed as soil conditioner/fertilizer, are other remarkable
78 contamination routes. These biosolids can contain appreciable amounts of residual drugs [12, 13]. Despite FQs
79 photosensitivity, both in water [14, 15] and soil systems [16, 17], the presence of these antibiotics in the environment
80 implicates serious threats to the ecosystem and human health. Mainly, bacterial resistance stimulation [18, 19] and
81 formation of pharmacologically active photoproducts that contribute to the overall impact [19, 20]. For these reasons,
82 monitoring FQs in the environment is an important task, and needs accurate and sensitive analytical methods. The low
83 concentrations usually detected in natural waters (from tens ng L⁻¹ to few μ g L⁻¹) require a SPE pre-concentration step.
84 In this work we prepared a novel SPE sorbent from pyrolysis of Kraft lignin (LG) onto silica. LG was chosen as the
85 carbon precursor due to its base aromatic structure, large availability and low cost. LG is indeed one of the three main
86 constituents of biomass together with cellulose and hemicelluloses, and also the major waste of the pulping process of
87 the paper industry, with increasing amounts from the emerging cellulosic ethanol industry [21, 22]. As apparent from
88 the new insights in LG pyrolysis [23, 24], growing attention is being paid to its conversion into renewable energy

89 sources by pyrolytic treatment, that yields valuable byproducts, *viz.* char, bio-oil, syngas, and fine chemicals such as
90 oxygenated aromatic compounds [25].

91 In particular, Kraft LG is obtained as a byproduct of the paper pulping process. In the Kraft process the insoluble LG
92 polymer present in wood is decomposed into smaller units under high temperatures (170-180°C) in the presence of
93 strong alkali solutions and sulfides. During the process the LG polymer backbone is broken and sulfides are included in
94 the structure, making it water-soluble.

95 LG pyrolysis, *i.e.* the treatment at high temperature in oxygen-free atmosphere, leads to the elimination of sulfur and
96 oxygen present in the polymer, thus leaving carbon as a residue [23, 25]. Indeed, from the chemical viewpoint LG is
97 one of the most interesting natural polymers for production of graphenes. In fact, LG backbone consists of
98 hydroxyphenylpropanoic units linked through ether and C-C condensed types of linkages, with *p*-coumaryl alcohol,
99 coniferyl alcohol, and sinapyl alcohol as the main monomers [25], that shows a polymerization degree of the order of
100 several hundred [26]. This complex base-structure has in principle great potentiality to be rearranged in extended
101 graphene-like aromatic layers, as demonstrated under microwave plasma irradiation [27] and also by pyrolytic treatment
102 [28]. It is documented that carbonaceous residues from LG pyrolysis contain appreciable amounts of polycyclic
103 aromatic hydrocarbons [25, 29], and that polymeric carbon with fine sorption properties can be obtained by pyrolysis of
104 olive stones LG biomass [26]. It is also reported that softwood pyrolysis yields graphene sheets rearranged in
105 turbostratic carbon [28], and recently it was seen that pyrolysis of LG deposited on SiO₂/silicon substrates produces
106 graphene-like carbons [30]. Furthermore, it is well known that pyrolysis of Kraft LG proceeds in two steps according to
107 the pyrolysis temperature. Between 200 and 400°C CO₂ is produced together with volatile sulfur compounds [23, 31],
108 and in the range 500-600°C CO is evolved from phenols decomposition. H₂ production begins at 500°C [32, 33],
109 indicating that, starting from this temperature, carbon defects are cured. In fact, bulk pyrolysis of LG at high
110 temperature (> 3000 K) is known to yield high-ordered carbon [34].

111 Herein we report the preparation of a carbon-based material consisting of silica-supported pyrolyzed LG, to be used as a
112 novel SPE sorbent. The preparation of the material, simple and costless, consists of two steps only, *viz.* adsorption of
113 aqueous LG onto micrometric silica followed by pyrolytic treatment to convert lignin into sp² carbon phase. LG-silica
114 has been characterized by thermogravimetric analysis (TGA), scanning electron microscopy (SEM), surface area BET
115 measurements and electron paramagnetic resonance (EPR), and tested as SPE sorbent for pre-concentration of
116 Marbofloxacin (MAR) and Enrofloxacin (ENR) from tap and not tampered river water. These two FQ drugs, widely
117 diffused in surface waters of Northern Italy due to the large use for veterinary purposes [35], were chosen as probe
118 contaminants to test this novel sorbent as extractant. After SPE on LG-silica, separation and quantification were

119 performed by high performance liquid chromatography coupled with fluorescence detection (HPLC-FD). The batch-to-
120 batch reproducibility was assessed, the main analytical aspects were evaluated and, finally, the sorbent phase was
121 applied to the determination of FQs in actual environmental waters.

122

123 **Experimental**

124 *Chemicals*

125 All chemicals were reagent grade or higher in quality (the complete list is in Supplementary material).

126

127 *Preparation and characterization of LG-silica*

128 LG-silica was prepared by adding 1 g silica and known amounts (20-50-100 mg, nominal loadings 2-5-10 wt%) of LG
129 in 50 mL distilled water. The suspension was mixed to promote LG adsorption onto silica microparticles and then water
130 was removed under vacuum overnight. For the pyrolysis (high-temperature annealing under vacuum), 200 mg sample
131 was put in an alumina combustion boat, located inside a quartz tube inserted into a cylindrical oven (Watlow ceramic
132 fiber heater, i.d. 38 mm, length 305 mm, electrical power 600 W). A double stage diffusion and rotary vacuum pump
133 was used to work in vacuum (10^{-3} Pa), mandatory to get anoxic atmosphere. The heating rate of the oven was $10^{\circ}\text{C min}^{-1}$
134 and the final temperature of 1200°C was kept for 20 min.

135 The final material was characterized by SEM, BET, TGA and EPR (experimental details in Supplementary material).

136

137 *SPE procedure*

138 The cartridge (6-mL polypropylene tubes) was prepared by slurry packing 200 mg LG-silica, dispersed in MeOH,
139 between two polyethylene frits. To remove impurities and minimize void/channeling effect the cartridge was washed
140 under vacuum (Resprep manifold, Restek Corporation, Bellefonte, USA) with 40 mL MeOH, 40 mL distilled water, 10
141 mL 50 mM TBAH-ACN (70:30) and finally 40 mL distilled water. Before the extraction procedure, performed by a
142 multi-channel peristaltic pump (Gilson, Italy), the cartridge was conditioned with 5 mL MeOH and 5 mL ultrapure
143 water; the water sample (50-500 mL) was fed to the column at a flow rate of $\sim 3.5 \text{ mL min}^{-1}$, and then the cartridge was
144 dried for 5 min. The analytes were eluted under vacuum with $2 \times 2 \text{ mL ACN-50 mM TBAH (30:70)}$, at a flow rate of 1
145 mL min^{-1} . The extract was acidified with 1 M HCl (pH ~ 2.5) before HPLC injection. Details about water samples and
146 HPLC are in Supplementary material.

147

148 *Analytical evaluation*

149 Since the aim was applying LG-silica to real samples, the analytical evaluation of the whole procedure was carried out.
150 As no CRMs are available, trueness was evaluated by recovery tests [36, 37], on tap water fortified with different
151 amounts of FQs, in the range 20-1000 ng L⁻¹ (n=3), and on blank river water – previously analyzed by a published
152 procedure [35] – spiked at 10-100 ng L⁻¹ (n=3).

153 The intra-day precision (repeatability) was evaluated on independently fortified tap water samples (50 ng L⁻¹, n=3). The
154 inter-day precision was assessed on tap water (20-1000 ng L⁻¹, n=3) and also on river water spiked at 20-100 ng L⁻¹
155 (n=3).

156 The batch-to-batch reproducibility was studied by recovery tests on tap water (50 mL) spiked with 5 µg L⁻¹ of MAR and
157 ENR, using three LG-silica samples independently synthesized following the entire preparation.

158 Selectivity was evaluated from the HPLC-FD chromatograms of the SPE extracts obtained by pre-concentration of not
159 tampered blank river water [38].

160 Linearity was determined by three independent six-point calibration curves generated for each analyte in the range 1-10
161 µg L⁻¹ using the weighted linear least squares (wLLSR) regression.

162 Theoretical method detection and quantification limits (MDLs, MQLs) were obtained on the basis of the instrumental
163 detection and quantification limits (IDLs, IQLs) calculated using the residual standard deviation (Sy/x) of the wLLSR
164 regression as (3 × Sy/x)/slope and (10 × Sy/x)/slope, respectively. Experimental method detection and quantification
165 limits (MDL, MQL) were determined as the minimum concentration that provided a FD response significantly different
166 from zero, and the lowest concentration that provided acceptable recovery (≥70%) and precision (RSD<20%),
167 respectively [38].

168

169 **Results and Discussion**

170 *Characterization of LG-silica*

171 Fig. 1 shows the SEM images of pristine silica (a), LG-free silica after pyrolysis (b) and 2wt% LG@silica after
172 pyrolysis (c), together with the related pictures (a1,b1,c1). LG-silica appears as a blackish powder (c1), while the other
173 two samples appear white colored (a1,b1). SEM characterization indicates that in the pristine material (a) the particles
174 surface is smoother and the particle size is slightly bigger compared to the treated samples (b,c). Apart from these little
175 differences, no substantial changes in shape and dimension occurred after thermal treatment (b). Moreover, no
176 significant features can be found in LG-silica (c) compared to the samples not containing LG (a,b), reasonably due to
177 the small amount of immobilized carbonaceous material. Indeed, results from TGA showed that silica was effectively
178 derivatized with pyrolytic carbon, 0.72, 1.12 and 1.23wt% for the samples prepared with nominal LG loadings of 2, 5

179 and 10wt%, respectively. The residual carbon content resulted lower with respect to the initial loading due to the weight
180 loss occurring during pyrolysis (loss of oxygenated and sulfur moieties present in the Kraft lignin [23]).

181 BET analysis indicated surface area values of $370 \text{ m}^2 \text{ g}^{-1}$ and $430 \text{ m}^2 \text{ g}^{-1}$ for LG-silica and for the *blank* silica submitted
182 to the same pyrolytic treatment, respectively. Compared to pristine silica ($450 \text{ m}^2 \text{ g}^{-1}$), the value found for LG-silica
183 accounted for the silica surface modification induced by the deposition of LG-derived carbons.

184 The LG-silica samples were analyzed by EPR spectroscopy at ambient temperature (see Supplementary material). It is
185 evident that the spectrum shape is as function of pyrolysis temperature. The sample pyrolyzed at lower temperature (Fig.
186 S1) showed an asymmetric peak due to the presence of both carbon and oxygen centred radicals. Moreover, the
187 spectrum linewidth decreased at higher temperature due to the curing of the material with the formation of a less
188 defective graphitized carbon. Another information deriving from the EPR measurement is the estimation of the
189 extension of the delocalization of the free electrons on carbon. In fact, by increasing the applied microwave power, the
190 EPR active sites can undergo to a saturation due to the relaxation mechanism. More effective is the relaxation (thus
191 more extended is the delocalization) the lower is the saturation. From the analysis of the curves (Fig. S2) appears how
192 an higher temperature gives a less defective carbon (the number of spins per gram is lower), but with a formation of
193 smaller domains (increased deviation from the linearity at high power).

194

195 *Solid-phase extraction on LG-silica*

196 Preliminary tests were performed to investigate the affinity of the two drugs for LG-silica. The amount of material (200
197 mg) was chosen basing on the most used SPE sorbents commercially available for enrichment of trace contaminants
198 from surface waters, FQs included [9]. The first recovery tests were carried out on tap water (50 mL) spiked with $5 \mu\text{g}$
199 L^{-1} of each FQ ($n=3$), working at the native sample pH (7.7 ± 0.1). The percolated samples after SPE were analyzed by
200 HPLC-FD to verify the antibiotics adsorption. MAR and ENR were quantitatively adsorbed on the 2-5wt% LG-silica
201 ($\text{MAR} \leq \text{IDL}$ and $\text{ENR} < \text{IQL}$ in the percolated samples); in the case of 10wt% LG-silica MAR concentration was $<$
202 IQL , while just 8% of the ENR initial amount was not adsorbed (for IDL and IQL see *Analytical evaluation*). These
203 findings attest the good affinity of the zwitterionic form of the two drugs for LG-silica, accordingly to their dissociation
204 constants (MAR pK_{a1} 5.51-5.69, pK_{a2} 8.02-8.58; ENR pK_{a1} 6.27, pK_{a2} 8.3 [9]), and in agreement with the results
205 previously obtained on graphene-derivatized silica [8]. Therefore no pH adjustment before SPE was necessary for
206 molecular adsorption.

207 The role of LG-derived pyrolytic carbon in the adsorption of the two drugs was proved by testing silica *not* containing
208 LG (*blank* control sample) that was submitted to the same pyrolytic process. It was verified that the bare material does

209 not show any adsorption affinity for FQs, clearly highlighting the key role of LG immobilized onto the silica support by
210 pyrolysis, even at low wt%.

211 Considering the preliminary findings and TGA results, it seemed convenient to select 2wt% LG-silica (real carbon
212 content 0.72wt%), that was then tested for further extractions (50 mL tap water, native pH, spike $5 \mu\text{g L}^{-1}$) to investigate
213 the elution step. Basing on previous papers [8, 9] and on the material characteristics (i.e. type and amount of carbon),
214 25mM H_3PO_4 -ACN (85:15), 1% NH_3 -MeOH (70:30) and 50 mM TBAH-ACN (70:30) were selected as the potential
215 eluting solutions. After pre-concentration, the analytes were eluted by 2×2 mL of the first solution obtaining very poor
216 recovery (<2%), while the second one allowed greater but not quantitative desorption (65-70% for MAR, 42-63% for
217 ENR); using 2×2 mL 50 mM TBAH-ACN (70:30) the recovery was 79(3)% and 75(1)% for MAR and ENR,
218 respectively.

219 The batch-to-batch reproducibility was assessed by recovery tests on three batches of LG-silica (nominal 2wt%)
220 independently prepared starting from the pristine microparticles; SPEs of 50 mL tap water samples spiked with $5 \mu\text{g L}^{-1}$
221 gave RSD values of 8% and 7% for MAR and ENR, respectively, using 50 mM TBAH-ACN (70:30).

222 This solution was then tested for recovery tests performed at lower concentrations, from 20 to 1000 ng L^{-1} (sample
223 volumes up to 500 mL). Results showed that analytes were quantitatively eluted by 2×2 mL, obtaining satisfactory
224 recovery and precision (Table 1), and at the same time pre-concentration factors up to 125. These results obtained on
225 LG-derived carbon supported on silica (200 mg, measured carbon 0.72wt%) were really satisfactory compared to those
226 obtained using 200 mg of bulk HLB polymer [35, 39]. In view of the good performance of LG-silica, we tested its
227 extraction ability in a more complex matrix, that is not tampered river water. Recovery trials were undertaken at
228 concentrations in the range 10-100 ng L^{-1} , amounts commonly detected in natural-contaminated surface waters [9, 35].
229 As shown in Table 1, recovery was quantitative for concentrations between 20 and 100 ng L^{-1} , with values higher than
230 70% and RSDs below 14%. This indicates that LG-silica is a suitable sorbent for SPE of FQs in actual samples, and a
231 typical chromatogram is shown in Fig. 2. At the lowest spike (10 ng L^{-1}) recovery was anyway semiquantitative (see
232 Table 1).

233 From the results, it appears that derivatization of silica with LG-derived pyrolytic carbon is a valid new route to obtain a
234 composite SPE material with good adsorption properties. This is more clearly evident considering that the amount of
235 carbon in the final material is around 1 wt%. Therefore, 200 mg LG-silica containing ca. 2 mg pyrolytic carbon allowed
236 processing sample volumes up to 500 mL, providing pre-concentration factors suitable for environmental determination
237 at the low ng L^{-1} . These experimental evidences make this novel material certainly appealing compared to the

238 commercial bulk HLB polymer, but also to other silica-supported carbons such as C18-silica [35] and graphene-
239 derivatized silica [8]. The results here presented give new evidences about the great potentiality of low-cost materials
240 (i.e. waste lignin biomass) for analytical application.

241

242 *Analytical evaluation*

243 Before application of LG-silica to real samples, the main analytical aspects of the entire procedure were evaluated, as
244 hereafter summarized. Due to the absence of CRMs, trueness was evaluated by recovery tests. All the mean recoveries
245 (%) obtained in water samples spiked at environmentally significant concentrations (10-1000 ng L⁻¹) are gathered in
246 Table 1. Recovery was calculated as the ratio between the concentration determined in the SPE extract and that
247 expected after pre-concentration on LG-silica, calculated considering the initial amount of analyte and the enrichment
248 factor. As apparent, recovery was generally between 71% and 105% in tap water; quantitative extraction, with
249 recoveries higher than 70% ($n=3$), was also obtained in untreated river water spiked with 20-100 ng L⁻¹, and around
250 55% for the lowest concentration of 10 ng L⁻¹. Precision was evaluated by calculating the RSDs associated to the mean
251 recovery obtained for each concentration (see Table 1). The intra-day precision (repeatability), calculated for 50 ng L⁻¹
252 spike, showed RSDs <8% ($n=3$). As shown in Table 1, the inter-day precision showed RSDs <15% ($n=3$) in tap water,
253 for spikes in the range 10-1000 ng L⁻¹; suitable reproducibility was also observed in untreated river water spiked with
254 10-100 ng L⁻¹ FQs, obtaining RSDs lower than 16% ($n=3$).

255 Selectivity was checked from the chromatograms of blank water extracts. Fig. 2 shows the profile obtained for a blank
256 river water SPE extract and the one obtained from the same river water sample spiked with 50 ng L⁻¹ of each FQ
257 (enrichment factor 125). No peaks were noticed at the retention times of the two analytes, thus interference of matrix
258 substances accountable for false-positive signals can be excluded.

259 No matrix interference was observed at the FD [17] from the analysis of FQs standard solutions prepared in the SPE
260 eluting solution and in the SPE extracts from pre-concentration of blank river water (matrix-matched calibration), in
261 accordance with previous work [8]. The wLLSR regression equations obtained in 50 mM TBAH-ACN (70:30), mean of
262 three independent calibration lines, showed good linearity for both drugs, as reported in Table 2 together with
263 instrumental and method sensitivity. Theoretical MDL and MQL were in the range of few ng L⁻¹, while the MDL and
264 MQL in raw river water experimentally determined were respectively 8 ng L⁻¹ and 20 ng L⁻¹, for both compounds,
265 thanks to the enrichment factor gained using LG-silica. These values are comparable with those reported for FQs in
266 river water by SPE on HLB followed by UPLC-MS [40]. Considering the typical FQs concentrations in surface waters

267 [8, 9], the method sensitivity is suitable for quantitative determination in actual samples, but also for qualitative
268 “sensing” at the low ng L⁻¹ levels.

269

270 *Application of LG-silica to actual samples*

271 LG-silica was finally applied for the determination of MAR and ENR in ditch waters collected in the Pavia county
272 downstream pigs and cattle farms regularly using these veterinary drugs. Variable amounts of antibiotics were
273 determined (confirmed also by duplicate spike on the SPE extract), ranging from few to some tens ng L⁻¹. In particular,
274 the highest concentrations of FQs were found in Ditch-1 sampled directly in the farm effluents, wherein 72 ng L⁻¹ of
275 MAR and 76 ng L⁻¹ of ENR were found (RSDs<7%, n=3). The chromatographic profile obtained for Ditch-1 after SPE
276 on LG-silica is shown in Fig. 3. FQs were detected also in Ditch-2, although their concentrations were below MQLs
277 (chromatogram not shown).

278 These findings evidence that livestock farms are major source for surface water contamination, further supporting
279 previous data [8, 35], and call for more care in controlling the diffusion of such emerging pollutants in rural areas
280 devoted to livestock breeding.

281

282 **Conclusions**

283 This is the first-time reporting on the application of pyrolytic carbon from LG as SPE sorbent phase. A novel sp²
284 carbonaceous material obtained from a low-cost biomass and supported on silica microparticles has been prepared,
285 characterized and tested as SPE cartridge for pre-concentration of FQ antibiotics from water, chosen as probes organic
286 contaminants to test the performance of the material. LG-silica, easily prepared in laboratory by pyrolysis of silica-
287 adsorbed LG, was packed in conventional SPE columns and proved to be useful for analytical application. In fact, pre-
288 concentration of sample volumes up to 500 mL provided enrichment factors up to 125, suitable for determination at
289 nanograms *per* liter levels. Quantitative recovery and suitable reproducibility were observed both in tap and untreated
290 river water. LG-silica was successfully employed for the analysis of actual surface water samples.

291 In conclusion, the preparation of this material is environmentally benign, simple, inexpensive and LG-silica is efficient
292 for pre-concentration of trace contaminants from aqueous complex matrices.

293

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300

301 The Authors declare that they have no conflict of interest.

302

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445 **Figure captions**

446 **Fig. 1** SEM images acquired on pristine silica (a), pyrolyzed bare silica (b), pyrolyzed LG@silica (c), and their related
447 pictures (a1,b1,c1)

448

449 **Fig. 2** HPLC-FD chromatograms overlay of a blank river water (500 mL) SPE extract (a) and the SPE extract of the
450 same river water sample spiked with 50 ng L⁻¹ FQs prior pre-concentration (b)

451

452 **Fig. 3** HPLC-FD chromatogram obtained from pre-concentration of the Ditch 1 sample collected in a livestock farming
453 area near Pavia (Lombardy, Italy)

454

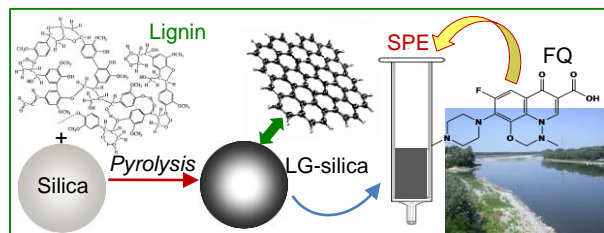
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456 **Table captions**

457 **Table 1** Mean recoveries (%) and inter-day precision RSDs (%) for LG-silica SPE of tap and river water samples
458 spiked with FQs

459

460 **Table 2** Calibration curves, correlation coefficients, detection and quantification limits





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To the Editor of **Microchimica Acta**, Prof. Wolfbeis

Dear Editor,

we are sending a paper on the preparation, characterization and analytical application of silica microparticles supporting carbons from Kraft lignin pyrolysis. The material was tested as solid-phase extraction sorbent for pre-concentration of Fluoroquinolones, chosen as model emerging contaminants, from natural waters. Please find enclosed the manuscript we wish to submit for publication in **MCA** as an **Original Paper**.

Manuscript title

Silica-supported carbon from Kraft lignin pyrolysis for solid-phase extraction of fluoroquinolone probes pollutants from environmental waters

Manuscript significance

This work presents the application of a novel carbon-based material, made of silica-supported pyrolytic carbon deriving from lignin, for solid-phase extraction (SPE) of emerging contaminants from water, specifically Fluoroquinolone antibiotics (FQs), chosen as test compounds.

A hybrid material consisting of silica microparticles supporting pyrolytic sp^2 carbon, easily obtained from Kraft lignin as the carbon precursor, was prepared, characterized by various techniques (SEM, TGA, BET, EPR) and evaluated for pre-concentration of Marbofloxacin (MAR) and Enrofloxacin (ENR), two drugs largely used in veterinary medicine, chosen as the probe pollutants.

To the best of our knowledge, this is the first report on the application of a carbon-based material obtained by lignin pyrolysis for SPE purposes.

The material (LG-silica) was tested on tap and river water samples spiked with MAR and ENR at concentrations in the range 10-1000 ng L⁻¹. Quantitative adsorption was observed at the sample native pH for both drugs, which were eluted from the cartridge with 2 × 2 mL aqueous tetrabutyl ammonium hydroxide-acetonitrile (70:30), and quantified by HPLC-FD. Quantitative recoveries (70-116%, $n=3$), and suitable inter-day precision (RSDs 8-16%, $n=3$) were observed for 20-1000 ng L⁻¹ spikes, also in raw river water. Sample volumes up to 500 mL provided enrichment factors up to 125, useful for FQs determination at environmentally significant levels (few ng L⁻¹), and at the same time attractive compared to the commercial sorbents. The batch-to-batch reproducibility was verified, and the material was successfully applied to the determination of these drugs in actual environmental waters.

In conclusion, the new sorbent phase is easy to be prepared, costless, efficient and applicable for quantitative determinations in real contaminated water samples. Results suggest that use of LG-silica could be extended also to other classes of contaminants.

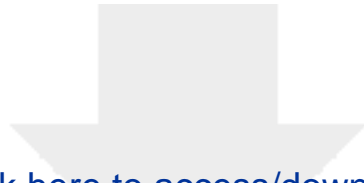
The paper is unpublished and not submitted for publication elsewhere. The authors declare no conflict of interest.

Best regards

Andrea Speltini and co-workers

Andrea Speltini

Pavia, January 3, 2016



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Figure 1

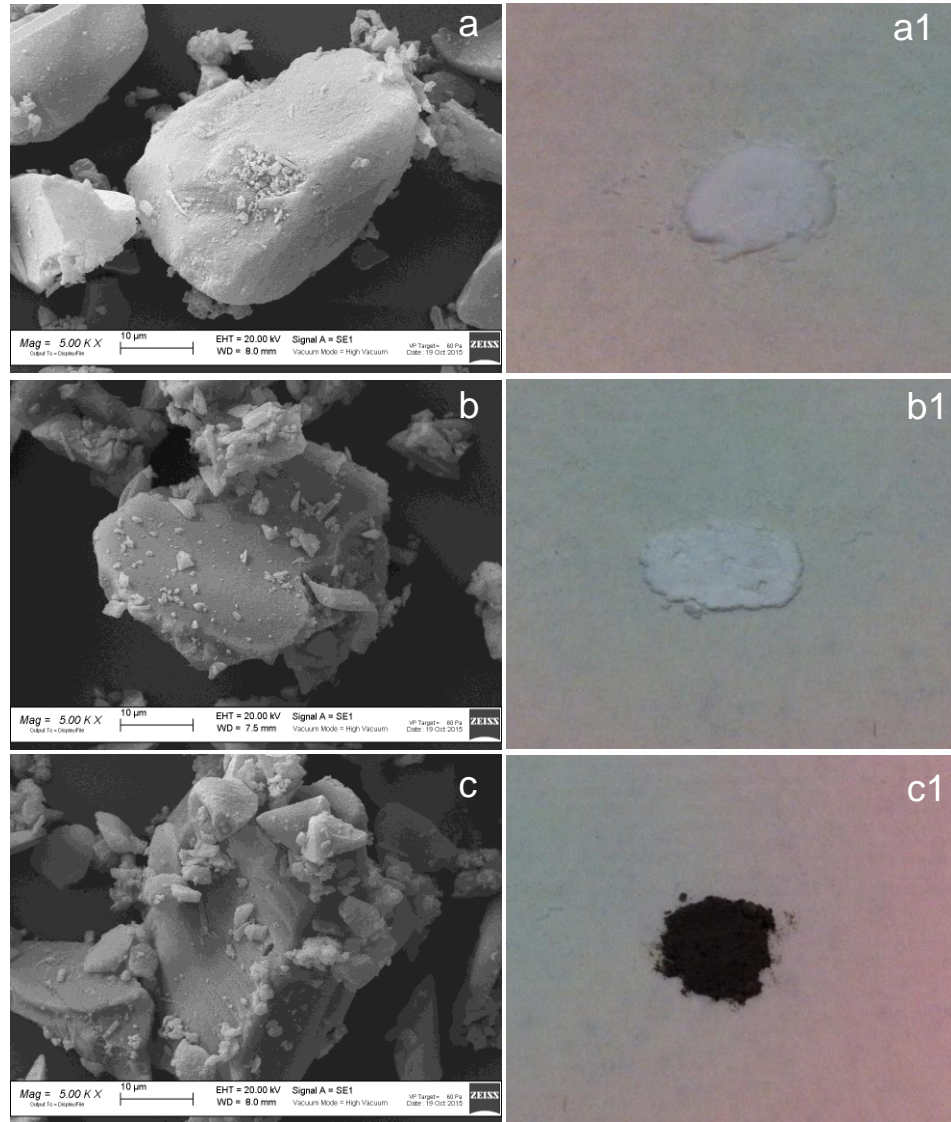


Figure 2

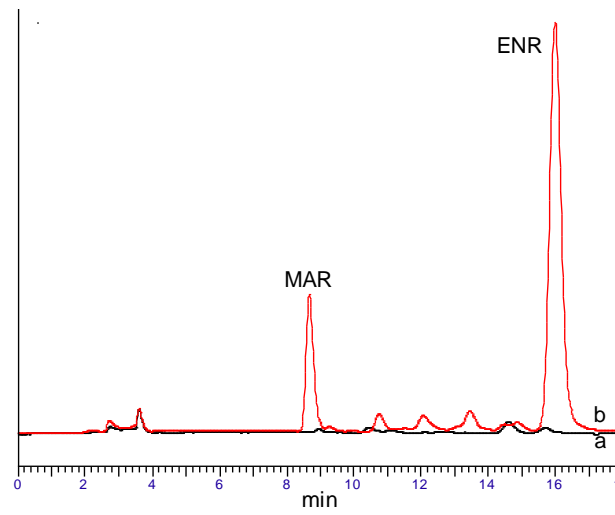


Figure 3

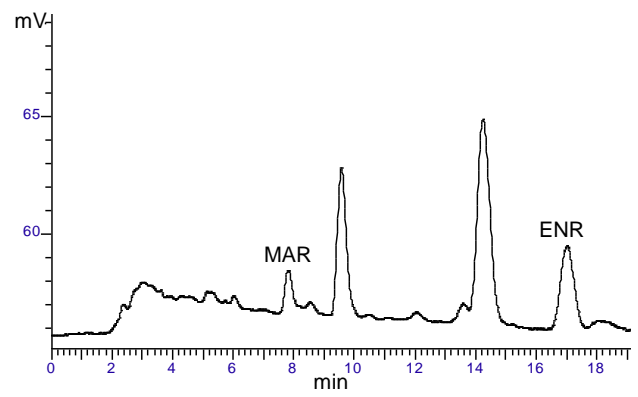


Table 1

Mean absolute recovery (%) and inter-day precision (RSD%)								
	Tap water (<i>n</i> =3)				River water (<i>n</i> =3)			
<i>Spike ng L⁻¹</i>	25	50	500	1000	10	20	50	100
MAR	73(16)	81(8)	71(9)	105(11)	51(15)	115(11)	116(10)	75(9)
ENR	92(12)	90(7)	93(10)	90(13)	56(7)	72(14)	70(9)	77(9)

Table 2

	Equation ^a	Linearity (r^2)	IDL ^b ($\mu\text{g L}^{-1}$)	IQL ^b ($\mu\text{g L}^{-1}$)	MDL ^b (ng L^{-1})	MQL ^b (ng L^{-1})
MAR	$y=18580(297) x - 342(425)$	0.9975	0.14	0.42	1.1	3.4
ENR	$y=171802(2307) x + 2073(3243)$	0.9982	0.11	0.34	0.9	2.7

^a Calculated as peak area (y) vs. analyte concentration (x); in parentheses slope and intercept uncertainties obtained by wLLSR (weighted linear least squares regression).

^b Calculated from wLLSR parameters.