

Analytical Methods

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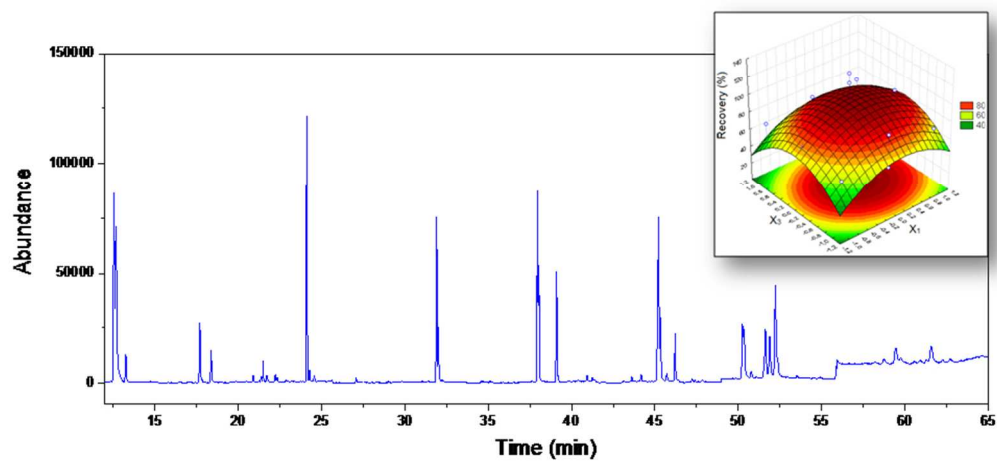


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1 **Box-Behnken design applied to ultrasound-assisted extraction for the**
2 **determination of polycyclic aromatic hydrocarbons in river sediment by gas**
3 **chromatography/mass spectrometry**

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14

15 **ABSTRACT**

16 This paper describes the optimization of the extraction of polycyclic aromatic
17 hydrocarbons (PAHs) in sediment samples from a river by applying an ultrasonic bath
18 for quantification by gas chromatography/mass spectrometry (GC/MS). The factors n-
19 hexane and dichloromethane proportion in the solvent mixture, solvent mixture volume
20 and sonication time were optimized to take into account a 3³ Box-Behnken design with
21 a triplicate central point. The optimum extraction conditions achieved through a
22 response surface methodology study consisted of 15 minutes of sonication time and 10
23 mL of solvent mixture composed of 55% of n-hexane. The optimized conditions were
24 validated by assessing the percentage recovery obtained for blank spike and sample
25 spike using as solvent mixture n-hexane/dichloromethane (55:45) and n-hexane/acetone
26 (55:45). The extraction with n-hexane/acetone (55:45) presented lower percent relative
27 standard deviation and greater efficiency in the extraction process, since hexane/acetone
28 (55:45) led to higher mean percentages of recovery of the blank spike and the sample
29 spike.

30 **KEYWORDS:** Ultrasonic bath, PAHs, Sediment, Box-Behnken design.

31

32 1. INTRODUCTION

33 Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous organic
34 pollutants and their presence has been studied in different matrices such as water,
35 atmospheric particulate matter, and marine and river sediments.^{1, 2} Some of them are
36 known to be carcinogenic or mutagenic because they are metabolized by *hydrocarbon*
37 *hydroxylases* present in the liver and their derivatives bind to DNA inducing mutations
38 and cancers.^{3, 4} Thus, the PAHs were included in the lists of priority pollutants by the
39 Environmental Protection Agency of the United States (U.S. EPA), which are:
40 Acenaphthene, Acenaphthylene, Anthracene, Benzo[a]anthracene, Benzo[a]pyrene,
41 Benzo[b]fluoranthene, Benzo[ghi]perylene, Benzo[k]fluoranthene, Chrysene,
42 Dibenzo[a,c]anthracene, Fluoranthene, Fluorene, Indeno[1,2,3-cd]fluoranthene,
43 Naphthalene, Phenanthrene, Pyrene (16 U.S. EPA PAHs).⁵ Since these compounds are
44 highly lipophilic and have low biodegradability, they are generally found adsorbed to
45 particulate material rich in organic matter, mixing with minerals of various types and
46 granules present on the bottom of rivers, participating in the formation of sediment.^{1, 6}

47 Several methods have been described for the determination of PAHs in
48 environmental samples, with different techniques of extraction, purification and
49 detection, according to the matrix. For soil and sediment samples, Soxhlet extraction is
50 the recommended method by the U.S. EPA and by National Oceanic and Atmospheric
51 Administration (NOAA).^{7, 8} This is the most commonly used method as the extraction is
52 simple, but has some disadvantages, such as that it is time-consuming and requires large
53 volumes of organic solvents.⁹ To reduce the extraction time and solvent consumption,
54 other techniques have been applied, among them accelerated solvent extraction,
55 supercritical fluid extraction and microwave-assisted extraction. However, besides

56 having a high cost, the extraction efficiency for these techniques are directly related to
57 the type of matrix.⁹⁻¹¹ Thus, ultrasound can be highlighted because of its low cost,
58 small volume of organic solvents, simplicity of operation and because it provides safer
59 conditions for the analyst, since the methods enable operation at ambient temperature
60 and pressure.^{9, 10}

61 Several studies have shown that sonication provides a similar or higher
62 extraction efficiency of PAHs in sediment and soil samples compared with other
63 extraction techniques. In a comparative study, Marvin *et al.* (1992)¹² concluded that
64 Soxhlet and ultrasound methods presented equal extraction efficiency, and also that the
65 ultrasound extraction was faster (45 minutes) than Soxhlet extraction (48 hours).
66 Likewise, Sun *et al.* (1998)¹³ evaluated the efficiency of extraction for the 16 U.S. EPA
67 PAHs in soil samples for ultrasonic extraction using different solvents and verified that
68 sonication using acetone gave better results than Soxhlet extraction. Banjoo *et al.*
69 (2005)¹⁴ optimized an ultrasonic extraction using n-hexane–acetone (1:1) mixture and
70 concluded that this method resulted in better precision and comparable quantities of
71 individual PAHs when compared to the reflux procedure using methanolic potassium
72 hydroxide . Tuncel and Topal (2011)¹⁵ performed a screening analysis to evaluate the
73 factors that may influence the extraction efficiency for PAHs in sediment samples for
74 three different extraction techniques: Soxhlet, ultrasonic bath and solid-phase micro-
75 extraction (SPME), and the best extraction method chosen was the ultrasonic bath .

76 In extraction, some interfering compounds could be co-extracted from
77 matrix, hence the cleanup step for chromatographic analysis is essential to
78 reduce those interferences. Adsorption purification using solid phase, including
79 chromatography column with silica gel/alumina and solid-phase extraction (SPE), is the
80 most used method.^{8, 16, 17}

81 Taking into account the works reported in the literature using an ultrasonic bath
82 for PAH sediments sample preparation, it was observed that there is scope for new
83 analytical considerations. Thus, the present work aimed to optimize a faster extraction
84 process under low solvent consumption for the 16 U.S. EPA PAHs in sediment samples
85 from a river using an ultrasonic bath. To develop a method for ultrasound extraction,
86 some parameters have to be optimized, such as solvent composition, volume and
87 extraction time. Generally, the optimization procedures are conducted one factor at a
88 time, which involves many experiments and is time-consuming and uses large volumes
89 of organic solvents. Therefore, the experimental design becomes a useful tool to
90 evaluate variables that influence the extraction process by means of a simultaneous
91 study of these variables carrying out a few experiments. Thus, in the present study a
92 new surface-response methodology was performed using a 3^3 Box-Behnken design to
93 optimize the factors time, organic solvent volume and solvent mixture composition for
94 ultrasound extraction.

95

96 **2. EXPERIMENTAL**

97 **2.1. Chemicals and solutions**

98 PAHs standard solution containing 16 U.S. EPA PAHs ($4000 \text{ ng } \mu\text{L}^{-1}$) and a
99 solution of surrogate PAHs ($2000 \text{ ng } \mu\text{L}^{-1}$; naphthalene-d8, acenaphthene-d10,
100 phenanthrene-d10, chrysene-d12 and perylene-d12) were supplied by Accustandard
101 (purity > 98%). Internal standard fluoranthene-d10 was supplied by Sigma-Aldrich
102 (purity 98 %). The solvents dichloromethane (DCM) and n-hexane (n-HEX) were all
103 pesticide grade, and acetone was HPLC grade.

104 The calibration stock solution of 16 U.S. EPA PAHs was prepared at a
105 concentration of $10 \text{ ng } \mu\text{L}^{-1}$ in dichloromethane and was diluted to working

106 concentrations of 0.1, 0.25, 0.50, 0.75, 1.00 and 1.25 ng μL^{-1} . The surrogate standards
107 and internal standard was added to a resulting concentration of 0.75 ng μL^{-1} in all
108 standard solutions.

109

110 **2.2. Sample extraction**

111 Extraction optimization was performed using samples of sediments from
112 Paraibuna river (Juiz de Fora, Brazil). Before extraction, an aliquot of surrogate
113 standard solutions was added to dry sediment to a resulting concentration of 75 ng g^{-1}
114 dry weight for each compound. The sediment samples were extracted in an ultrasonic
115 bath (Unique, USC2850 model) operating at a frequency of 25 kHz and power of 120
116 W. The extracts were concentrated to 1 mL using rotary evaporator and fractioned using
117 silica gel/alumina column with 3.2 g of silica gel and 2 g of alumina, both deactivated 5
118 % (w/w) with deionized water. The chromatographic column was eluted under gravity
119 with 16 mL of n-hexane/DCM (8:2). The eluate was then concentrated, the internal
120 standard was added and then the volume was adjusted with n-hexane to 1 mL. An
121 aliquot of 1 μL was injected into GC/MS.

122 Following the extraction procedure described above, the factorial design
123 optimization was performed using 10 g of sediment sample spiked with 16 U.S. EPA
124 PAHs, at a final concentration of 50 ng g^{-1} , for each compound, within the dry sediment.
125 The same procedure was used for recovery studies, with exception that a final
126 concentration of 75 ng g^{-1} , for each compound, was used for dry sediment. Sodium
127 sulphate anhydrous was used as blank, also with a final concentration of 75 ng g^{-1} dry
128 weight, for each compound.

129

130

131 **2.3. GC/MS apparatus**

132 The PAH analyses were conducted using an SHIMADZU GCMS-QP2010 plus,
133 equipped with autosampler PAL and an Rtx-5MS column (30 m, 0.25 mm i.d., 0.25- μ m
134 film thickness). The GC temperature program was: from 60°C to 80°C at 20°C min⁻¹,
135 then 2°C min⁻¹ to 103°C (1 min), then 5°C min⁻¹ to 280 °C (13 min) and 5°C min⁻¹ to
136 285°C (17 min). Helium ultrapure was used as carrier gas with a flow rate of 1 ml min⁻¹.
137 The mass spectrometer was operated in the selected ion-monitoring mode with electron
138 impact ionization voltage of 70 eV. Data acquisition and processing were accomplished
139 with the CGMSsolutions software.

140

141 **2.4 Software**

142 All statistical analyses were performed in Microsoft Office® Excel 2007 software,
143 while the response surfaces were performed in Statistica 6.0 software.

144

145 **3. RESULTS AND DISCUSSION**

146 **3.1. Optimization of ultrasound-assisted extraction**

147 The solvents used in extraction were selected by considering the solubility of
148 analytes and polarity. The principal methods of extraction of PAHs in sediment samples
149 use a solvents mixture composite of n-hexane and DCM in a proportion of 1:1. ^{7,8} There
150 are no descriptions in the literature of a study of these proportions, and this is very
151 important, since perhaps the ratio in the solvents mixture of DCM can be reduced. Thus,
152 in this study, the proportion of n-hexane and DCM was a selected variable. Another two
153 variables that can also affect the extraction efficiency were selected for study: the
154 volume of solvent mixture and sonication time in the ultrasonic bath. To optimize the
155 extraction process, a 3³ Box-Behnken design with a triplicate at the central point was

156 used. Box-Behnken design can be understood as a special fractional factorial design
 157 containing three-levels and k factors ($3k$, where $k \geq 3$), which allows the efficient
 158 estimation of the first- and second-order coefficients of the mathematical model. These
 159 designs are more efficient and economical than their corresponding $3k$ designs, mainly
 160 for a large number of variables, because they require an experiment number according
 161 to $N = 2k(k-1) + cp$ (cp means number of central points, which they are used to
 162 calculate the experimental error). Thus, all factor levels have to be adjusted only at three
 163 levels ($-1, 0, +1$) with equally spaced intervals between these levels.¹⁸ Table 1 shows
 164 the Coded 3^3 Box-Behnken Matrix containing, levels, factors and response (average
 165 percentage recovery) obtained for 16 PAHs for each test. For each experiment, a sample
 166 spiked to a final concentration of 50 ng g^{-1} dry weight of each of the 16 U.S. EPA PAHs
 167 was extracted.

168 **Table 1:** Coded 3^3 Box-Behnken Matrix containing, levels, factors and response (%
 169 average recovery) obtained for 16 U.S. EPA PAHs.

Issue	X_1	X_2	X_3	Recovery (%)
1	-1	-1	0	59.07
2	+1	-1	0	86.89
3	-1	+1	0	67.33
4	+1	+1	0	66.75
5	-1	0	-1	60.86
6	+1	0	-1	71.15
7	-1	0	+1	66.46
8	+1	0	+1	72.88
9	0	-1	-1	87.75
10	0	+1	-1	51.17
11	0	-1	+1	74.43
12	0	+1	+1	42.93
13	0	0	0	129.46
14	0	0	0	83.85
15	0	0	0	117.64

170 X_1 : Composition of mixture n-Hex/DCM (v/v): (-1): 25:75; (0): 50:50; (+1):

171 75:25

172 X_2 : Sonication time (min): (-1): 10; (0): 20; (+1): 30

173 X_3 : Volume of solvent mixture (mL): (-1): 08; (0): 10; (+1): 12

174

175 The response data obtained by the average recovery values of 16 U.S. EPA
 176 PAHs, by each experiment, are given in Table 1. Using a fitted full quadratic model
 177 (Equation 1), a response surface regression analysis using coded units was performed
 178 for each response factor.

$$179 \quad \hat{y} = \hat{\beta}_0 + \sum_{i=1}^k \hat{\beta}_i x_i + \sum_{i=1}^k \hat{\beta}_{ii} x_i^2 + \sum_{i < j} \beta_{ij} x_i x_j + r_i \quad (1)$$

180 Table 2 shows the calculated values for coefficients and p-values (p-value is the
 181 probability of the null hypothesis). Using a 5% significance level, a factor is considered
 182 to affect the response if the coefficients differ significantly from zero and the p-value <
 183 0.05. Thus, an evaluation of coefficients was made for each parameter and their
 184 interactions. Calculated p-values indicates that the three considered variables do not
 185 have a statistically significant effect on the response (p-value > 0.05). Moreover, the fit
 186 models were evaluated (ANOVA) and the results found indicated that no evidence of
 187 lack of fit was observed in the 95% confidence interval (p-value > 0.82).

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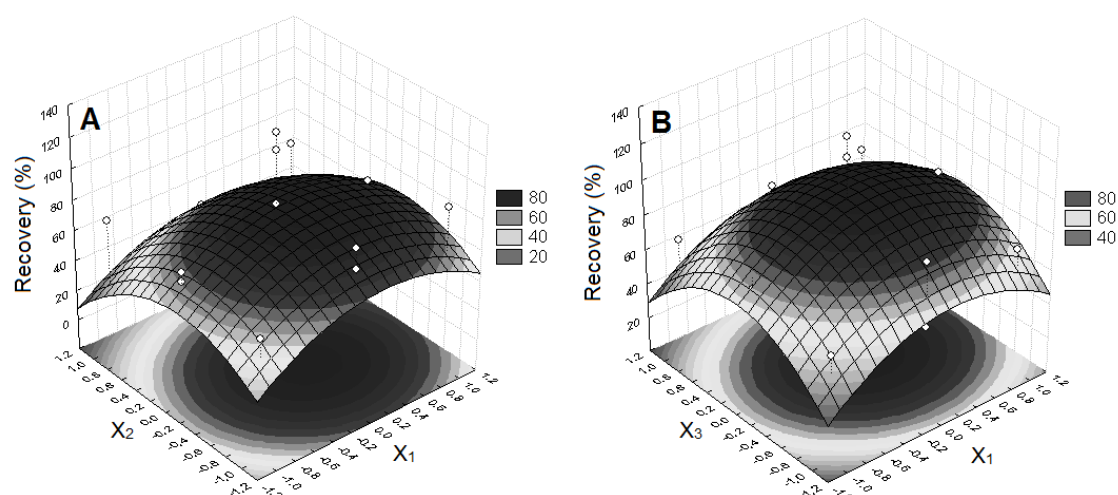
189 **Table 2:** Values obtained for coefficients, standard error, t-test and p-values with a 3³
 190 Box-Behnken design.

Coefficients	Regression coeff.	Standard error	t (2)	p-value
Mean	110.32	13.67	8.071	0.015
b ₁	5.49	8.37	0.656	0.579
b ₂	-9.99	8.37	-1.194	0.355
b ₃	-1.78	8.37	-0.213	0.851
b ₁₁	-18.27	12.32	-1.483	0.276
b ₂₂	-22.04	12.32	-1.789	0.216
b ₃₃	-24.21	12.32	-1.965	0.188
b ₁₂	-7.10	11.84	-0.600	0.609
b ₁₃	-0.97	11.84	-0.082	0.942
b ₂₃	1.27	11.84	0.107	0.925

191 To select the best extraction conditions, two response surfaces were performed
192 (Figure 1 A-B). At both the sonication time was fixed and the ratio of n-hexane (Figure
193 1A), and the solvent mixture volume (Figure 1B), was varied at each.

194 In both response surfaces, there was an optimal region for extraction of PAHs
195 localized between 13 to 22 min and between 14 to 24 min (Figures 1 A and 1 B,
196 respectively). Since there is a coincident time interval between the two surfaces, it is
197 possible to select a condition within this region that provides better recovery values
198 combined with higher throughput. Thus, the extraction condition selected involves three
199 sonication steps of 15 min with 10 mL of a mixture solvent composed of n-
200 hexane/DCM (55:45).

201



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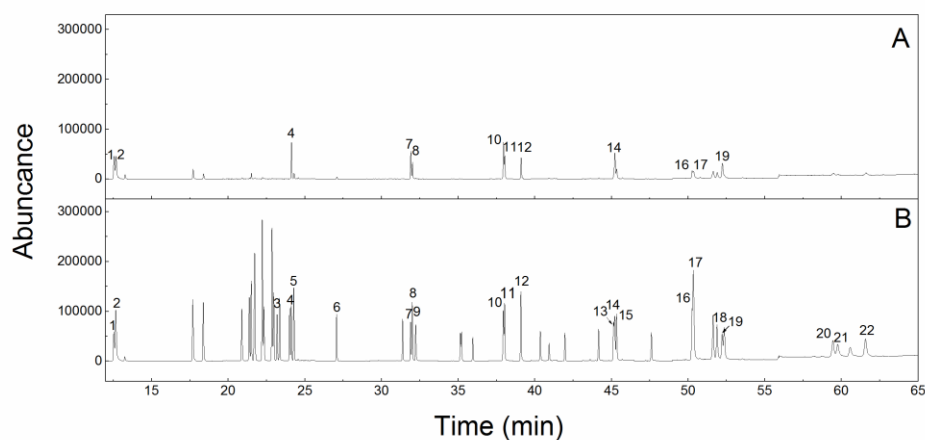
203 **Figure 1:** Response surfaces estimated from the Box-Behnken 3^3 design for
204 optimization of extraction conditions in an ultrasonic bath. **A:** Interaction between
205 sonication time and the ratio of n-hexane; **B:** Interaction between sonication time and
206 volume of solvent mixture.

207

208 **3.2. Recovery tests**

209 The extraction conditions selected by factorial design have been validated by
210 studying the recovery of the sample spike (n = 4) and the blank spike (n = 4), both with
211 a standard mixture containing the analytes with a final concentration of 75 ng g⁻¹ dry
212 weight. The main objective of this work was the determination of the 16 U.S. EPA
213 PAHs. However, the extraction method was optimized to be applied to analysis of these
214 and another 29 PAHs, including methylated compounds, which can be used to obtain
215 additional information regarding the origin of PAHs. ⁶ Figure 2 shows the
216 chromatograms obtained for the sample (A) and sample spike (B) extracted using the
217 selected conditions, in which only the 16 U.S. EPA PAHs are highlighted.

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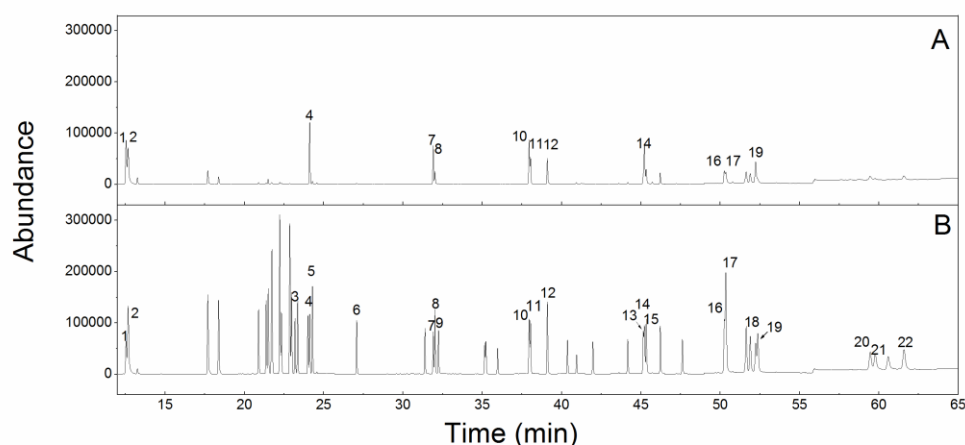
220 **Figure 2:** Chromatograms obtained for sample (A) and sample spike (B) extracted with
221 n-hexane/DCM (55:45). Peaks: 1) Naphthalene-d8, 2) Naphthalene, 3) Acenaphthylene,
222 4) Acenaphthene- d10, 5) Acenaphthene, 6) Fluorene, 7) Phenanthrene-d10, 8)
223 Phenanthrene, 9) Anthracene, 10) Fluoranthene-d10, 11) Fluoranthene, 12) Pyrene, 13)
224 Benz[a]anthracene, 14) Chrysene-d12, 15) Chrysene, 16) Benzo[b]fluoranthene, 17)
225 Benzo[k]fluoranthene, 18) Benzo[a]pyrene, 19) Perylene-d12, 20) Indeno[1,2,3-
226 cd]fluoranthene, 21) Dibenzo[a,c]anthracene, 22) Benzo[ghi]perylene.

227

228 Acetone was used to replace DCM in the solvent mixture, as chlorinated organic
 229 compounds are harmful to health and have a higher cost of disposal.⁷ Therefore, another
 230 study was conducted using the extraction conditions selected by factorial design but
 231 replacing the DCM with acetone in the solvent mixture. Thus, the sample spike (n = 4)
 232 and blank spike (n = 4), both with a standard mixture containing the analytes at a final
 233 concentration of 75 ng g⁻¹ dry weight, were extracted with a solvent mixture comprising
 234 n-hexane/acetone (55:45). Figure 3 shows the chromatograms obtained for the sample
 235 (A) and sample spike (B) extracted using this new mixture of solvents.

236 The recovery results for the 16 U.S. EPA PAHs obtained in the extraction
 237 sample spike and blank spike using the two solvent mixtures are shown in Table 3.

238



239 **Figure 3:** Chromatograms obtained for sample (A) and sample spike (B) extracted with
 240 n-hexane/acetone (55:45). Peaks: 1) Naphthalene-d8, 2) Naphthalene, 3)
 241 Acenaphthylene, 4) Acenaphthene- d10, 5) Acenaphthene, 6) Fluorene, 7)
 242 Phenanthrene-d10, 8) Phenanthrene, 9) Anthracene, 10) Fluoranthene-d10, 11)
 243 Fluoranthene, 12) Pyrene, 13) Benz[a]anthracene, 14) Chrysene-d12, 15) Chrysene, 16)
 244 Benzo[b]fluoranthene, 17) Benzo[k]fluoranthene, 18) Benzo[a]pyrene, 19) Perylene-
 245 d12, 20) Indeno[1,2,3-cd]fluoranthene, 21) Dibenzo[a,c]anthracene, 22)
 246 Benzo[ghi]perylene.

248

249 **Table 3:** Recovery and relative standard deviation (RSD) obtained for 16 U.S. EPA
 250 PAHs for extraction with an ultrasound bath of blank spike and sample spike using n-
 251 hexane/DCM (55:45) and n-hexane/acetone (55:45).

Compound	n-HEX/DCM (55:45)		n-HEX/Acetone (55:45)	
	Sample spike	Blank spike	Sample spike	Blank spike
Naphthalene	94(±4)	96(±14)	137(±7)	161(±10)
Acenaphthylene	80(±3)	77(±7)	76(±8)	83(±2)
Acenaphthene	78(±6)	88(±2)	87(±4)	91(±1)
Fluorene	90(±4)	91(±6)	89(±6)	105(±2)
Phenanthrene	68(±11)	86(±4)	97(±5)	92(±2)
Anthracene	71(±3)	77(±3)	79(±3)	80(±2)
Fluoranthene	62(±28)	97(±5)	102(±13)	112(±3)
Pyrene	81(±15)	97(±6)	109(±8)	112(±6)
Benz[a]anthracene	94(±12)	98(±3)	95(±7)	117(±2)
Chrysene	78(±12)	98(±2)	91(±5)	96(±1)
Benzo[b]fluoranthene	88(±10)	97(±13)	101(±2)	109(±2)
Benzo[k]fluoranthene	83(±6)	96(±4)	87(±7)	98(±1)
Benzo[a]pyrene	80(±11)	84(±3)	92(±1)	86(±2)
Indeno[1,2,3-cd]fluoranthene	78(±9)	70(±8)	83(±2)	103(±2)
Dibenzo[a,c]anthracene	94(±6)	97(±6)	109(±2)	116(±2)
Benzo[ghi]perylene	76(±9)	87(±3)	83(±3)	100(±3)

252

253 When examining trace-level compounds, the percentage recovery for the sample
 254 spike was in the range of 60% to 120%, which is acceptable, as are the values for the
 255 percent relative standard deviation (% RSD) between repetitions, which were less than
 256 30%.⁸

257 Both solvent mixtures exhibited satisfactory recovery percentage. The average
 258 recovery for the extraction with n-hexane/DCM (55:45) was 81% for the sample spike
 259 and 90% for the blank spike. The RSDs vary from 2% to 22% for the sample spike and
 260 3% to 25% for the blank spike. The values of average recovery from the sample spike
 261 provided by the response surfaces 1A and 1B were 84% and 82%, respectively. Thus,
 262 the value obtained experimentally was very close to the predicted value, indicating that
 263 the model obtained was valid to select a condition for extraction of PAHs in sediment

264 samples. For extraction with n-hexane/acetone (55:45), the average recovery values
265 were 95% and 104% for the sample and the blank spike, respectively. The values of
266 RSD varied from 1% to 13% for the sample spike and 1% to 10% for the blank spike.
267 Therefore, the extraction using n-hexane/acetone (55:45) resulted in a greater
268 percentage recovery of the analytes and a greater reproducibility between replicates, and
269 presented a lower RSD for most compounds.

270 According to Mitra (2003)¹⁹, the solvent selection depends on the nature of the
271 analytes and matrix, often using a mixture of water-miscible solvents (acetone) with
272 non-miscible ones (hexane or DCM). The water-miscible solvents can penetrate the
273 layer of moisture on the surface of the solid particles, facilitating the extraction of
274 hydrophilic organics. The hydrophobic solvents then extract the organic compounds of
275 like polarity, and so n-hexane is efficient in the extraction of nonpolar analytes, and
276 methylene chloride extracts the polar analytes. Therefore, when using a mixture
277 composed of n-hexane/acetone, the extraction of PAHs is favoured, since they have a
278 nonpolar character.

279 Thus, acetone can be used to replace DCM in the solvent mixture for the
280 extraction of PAHs in sediment samples, due to its greater efficiency in extraction of
281 analytes, as well as being a less costly (for commercial and disposal aspects) and less
282 toxic solvent.

283

284 **3.3. LOD and LOQ**

285 After selecting the solvent mixture for extraction, limit of detection (LOD) and
286 limit of quantification (LOQ) were determined. According to IUPAC (2002)²⁰, the
287 detection limit should be calculated as 3 times the standard deviation obtained for at
288 least 6 complete independent determinations of analyte at a typical matrix, blank or low-

289 level material. Thus, 10 g of sediment sample ($n = 6$) were spiked with 16 U.S. EPA
 290 PAHs, at a final concentration of 10 ng g^{-1} , for each compound, for dry weight. Spiked
 291 sediments were then extracted by three 15 min steps, using 10 mL of a solvent mixture
 292 composed of n-hexane/acetone (55:45). LOD and LOQ were calculated as $3s$ and $10s$,
 293 respectively, where s is the analysis standard deviation (Table 4). Method limits of
 294 detection ranged from 0.6 ng g^{-1} dry weight (Acenaphthylene) to 7.6 ng g^{-1} dry weight
 295 of (Benzo[a]anthracene), which are in accordance to literature.^{14, 21, 22} Method accuracy
 296 for low level concentrations (10 ng g^{-1}) was also evaluated by means of the percent
 297 relative standard deviation, with values not greater than 5%.

298

299 **Table 4:** Limit of detection (LOD), the limit of quantification (LOQ) and percent
 300 relative standard deviation ($n=6$) obtained for 16 U.S. EPA PAHs.

Compound	LOD (ng g^{-1} dry wt.)	LOQ (ng g^{-1} dry wt.)	RSD (%)
Naphthalene	5.5	18.5	2
Acenaphthylene	0.6	1.9	1
Acenaphthene	1.2	4.0	2
Fluorene	1.3	4.2	2
Phenanthrene	4.1	13.6	3
Anthracene	1.4	4.5	3
Fluoranthene	5.7	19.0	3
Pyrene	5.2	17.3	3
Benzo[a]anthracene	7.6	25.3	5
Chrysene	3.8	12.7	3
Benzo[b]fluoranthene	3.0	10.1	3
Benzo[k]fluoranthene	3.7	12.3	5
Benzo[a]pyrene	4.6	15.5	4
Indeno[1,2,3-cd]fluoranthene	2.1	6.9	3
Dibenzo[a,c]anthracene	1.6	5.2	3
Benzo[ghi]perylene	2.6	8.6	3

301 % RSD: percent relative standard deviation; dry wt.: dry weight.

302

303

304 4. CONCLUSION

305 Through a 3³ Box-Behnken design, it was possible to optimize a rapid method
306 (45 min) with low consumption of solvents for the extraction of PAHs in samples of
307 river sediment by ultrasound. The extraction conditions selected involve three steps of
308 sonication of 15 minutes with 10 mL of a mixture of solvents. Two solvent mixtures
309 were proposed for the extraction of PAHs in sediment samples, *i.e.* n-hexane/DCM
310 (55:45) and n-hexane/acetone (55:45). The extraction with a mixture composed of n-
311 hexane/acetone (55:45) was more efficient and reproducible, since it resulted in higher
312 percentages of recovery and lower percent relative standard deviation. Method limits of
313 detection ranged from 0.6 ng g⁻¹ dry weight (Acenaphthylene) to 7.6 ng g⁻¹ dry weight
314 of (Benzo[a]anthracene).

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