# High-throughput approach for the *in situ* generation of magnetic ionic liquids in parallel-dispersive droplet extraction of organic micropollutants in aqueous environmental samples

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## ABSTRACT

In this work, a novel and high-throughput parallel-dispersive droplet extraction (Pa-DDE) based on *in situ* formation of the hydrophobic MILs ($[Co(C_4IM)_4^{+2}]2[NTf_2^-]$, $[Ni(C_4IM)_4^{+2}]2[NTf_2^-]$ and $[Ni(BeIM)_4^{+2}]2[NTf_2^-]$) is demonstrated, for the first time, for the determination of benzophenone, metolachlor, triclocarban, pendimethalin, 4-methylbenzylidene camphor, and 2-ethylhexyl-4-methoxycinnamate from aqueous environmental samples. This experimental setup is comprised of a 96-well plate system containing a set of magnetic pins which were used to collect the MIL droplet after *in situ* formation. This consolidated system enabled simultaneous extraction of up to 96 samples and MIL production in one step. Using this apparatus, sample preparation times of 0.78 min per sample was achieved. The experimental conditions were carefully optimized using uni and multivariate approaches. The optimal conditions were comprised of sample volume of 1.25 mL, 4 mg of $[Co(C_4IM)_4^{+2}]2[Cl^-]$ and 40 µL of $LiNTf_2$ for the *in situ* formation, and dilution in 20 µL of acetonitrile. The analytical parameters of merit were successfully determined with LODs ranging from 7.5 to 25 µg $L^{-1}$ and coefficients of determination higher than 0.989. Intraday and interday precision ranged from 6.4 to 20.6 % (n = 3) and 11.6 to 22.9 % (n = 9), respectively, with analyte relative recovery ranging between 53.9 to 129.1 %.



*Keywords:* Magnetic ionic liquids; *in situ* formation; Parallel dispersive droplet extraction; Sample preparation; 96-well plate.

## 1. Introduction

Since the development of the microextraction techniques and the consolidation of the Green Analytical Chemistry (GAC), analytical methodologies have been directed towards creative solutions in order to avoid negative impacts to the human health and the environment [1,2]. In the sample preparation context, microextraction-based techniques are considered the greenest approach that fulfills the main aspects of the GAC principles [3] toward establishing good laboratory practices without hindering the analytical performance while maintaining efficiency and analyst safety [4,5]. Analytical chemists are consistently developing *green*, miniaturized and automated methodologies, prioritizing the named "3R" approach: **R**eduction of solvent volumes, **R**eplacement of harmful chemicals, and **R**ecycling [6]. For this reason, trends on reducing or eliminating toxic and volatile organic solvents have provided the introduction of a number of alternative solvents such as ionic liquids (ILs) and its derivatives [7,8].

Magnetic ionic liquids (MILs) are a subclass of ILs in which a paramagnetic component is incorporated to the IL structure, imparting magnetic susceptibility to the material. This is an important feature as it provides easier phase separation, which has been explored in various sample preparation methods [9–11]. Different types of cations and anions can produce MILs with varied physicochemical properties combined with unique solvation properties and negligible vapor pressure [9,12], which make them safer for the analyst and an interesting material for sample preparation. A number of applications of MILs in different sample preparation techniques have been reported for the determination of several compounds in biological, environmental and food samples by dispersive liquid-liquid microextraction (DLLME) [13–18], single drop microextraction (SDME) [19–21] and stir bar dispersive liquid microextraction (SBDLME) [22]. Moreover, this class of materials has recently been the subject of review articles [10,11].

The development of microextraction approaches based on the *in situ* formation of MILs is a recent and promising strategy. The *in situ* formation of ILs was proposed in 2009 [23] and its

development for MILs was possible due to the generation of novel solvents that contain the paramagnetic component in the cation, since it is not exchanged during the metathesis reaction [12,24]. Regarding the *in situ* process, a hydrophilic MIL, named the cation precursor (CP), is added to an aqueous sample solution. An anion exchange reagent (AER) is subsequently added allowing the mixture to undergo an *in situ* metathesis reaction, forming a hydrophobic MIL [9,24]. This reaction creates numerous finely dispersed hydrophobic MIL droplets, and the anion-exchange process increases the surface area of the extraction solvent, leading to high extraction efficiencies [25].

Few studies have exploited the *in situ* formation of MILs in microextraction approaches, which until now include the determination of organic contaminants in water by DLLME [24] and SBDLME [12]. Recently, DNA extraction was successfully performed through *in situ* MIL-DLLME [25]. These approaches have demonstrated the promise of the approach and exhibit advantages for the analytical procedure, as previously discussed.

Automation is also highly desirable in order to provide high throughput and reproducible analytical methodologies [26]. The 96-well plate system consists of an important tool in this scenario, since 96 samples can be processed at the same time [19,26–28]. Recently, our research group developed an apparatus for the use of MIL-SDME in combination with the 96-well plate system [19]. In this approach, named Parallel-single drop microextraction (Pa-SDME), neodymium rod magnets were adapted in the end of 96-well plate blades, which significantly increased the method throughput. Many previous generations of MILs contain the paramagnetic component in the anion (e.g., [$FeCl_3Br^-$], [$MnCl_4^{2-}$]), which presents challenges with regard to the *in situ* formation reaction since anions are the easiest to exchange to form the hydrophobic phase [24]. Moreover, MILs of this class require synthetic methods that employ organochlorine solvents as reaction media, which goes against the GAC principles.

In this study, a novel experimental strategy named *in sit*u Parallel-Dispersive Droplet Extraction (Pa-DDE) was developed and coupled with high-performance liquid

chromatography/diode array detection (HPLC-DAD) for the determination of benzophenone (BZP), metolachlor (MTC), triclocarban (TCC), pendimethalin (PDM), 4-methylbenzylidene camphor (4-MBC) and 2-ethylhexyl-4-methoxycinnamate (EHMC) in environmental water samples. These compounds were chosen as model analytes since they are considered micropollutants and their presence in the aquatic environment, even at low concentration, can provide risks to the environment and human health. Cobalt (II) and nickel (II) centers with imidazole ligands as cations and the bis[(trifluoromethyl)sulfonyl]imide ($[NTf_2^-]$) anion were selected as CP and AER, respectively. The experimental conditions were systematically optimized through univariate and multivariate approaches, and the analytical parameters of merit were obtained at the optimum conditions. To the best of our knowledge, this is the first report involving the *in situ* formation of hydrophobic MIL coupled to a 96-well plate system for microextraction purposes.

## 2. Experimental

### *2.1. Reagents and materials*

Analytical standards with high purity (≥98%) of benzophenone (BZP), metolachlor (MTC), triclocarban (TCC), pendimethalin (PDM), 4-methylbenzylidene camphor (4-MBC), and 2-ethylhexyl-4-methoxycinnamate (EHMC) and lithium bis[(trifluoromethyl)sulfonyl]imide ($[Li^+][NTf_2^-]$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile (ACN) and methanol (MeOH) were obtained from Merck (Kenilworth, NJ, USA) and ultrapure water (18.2 MΩ cm) was purified by a Mega Purity water purification system (Billerica, MA, USA). The pH adjustment was performed with a Britton-Robinson (BR) buffer solution 0.0500 mol $L^{-1}$, HCl and NaOH solutions of 3 mol $L^{-1}$ and 1 mol $L^{-1}$, respectively purchased from VETEC (Rio de Janeiro, RJ, Brazil). In order to produce CPs hydrated metal salts, 1-butylimidazole (98%) and 1-benzylimidazole (99%) were obtained from Sigma Aldrich (St. Louis, MO, USA). The hydrated metal salts were dried for at least four days at 50ºC.

Individual stock solutions of the analytes were prepared at concentrations of 10 and 1 g $L^{-1}$ in MeOH. In addition, a working solution containing a mixture of the analytes at concentration of 50 mg $L^{-1}$ was prepared by diluting appropriate amounts of the stock solution in ACN. Regarding the formation of the hydrophobic MILs, three different CP were evaluated as extraction solvents including tetrabutylimidazolenickel (II) chloride ($[Ni(C_4IM)_4^{2+}]2[Cl^-]$), tetrabenzylimidazolenickel (II) chloride ($[Ni(BeIM)_4^{2+}]2[Cl^-]$) and tetrabutylimidazolecobalt (II) chloride ($[Co(C_4IM)_4^{2+}]2[Cl^-]$); stock solutions of 10 g $L^{-1}$ of these compounds were prepared in ultrapure water and, solutions of 25 g $L^{-1}$ and 40 g $L^{-1}$ of $[Co(C_4IM)_4^{2+}]2[Cl^-]$ were prepared in ultrapure water. A working aqueous solution of $[Li^+][NTf_2^-]$ at 92 g $L^{-1}$ was also used for the experiments.

*2.2. Instrumentation*

A Shimadzu LC-20A system (Tokyo, Japan) comprised of a Rheodyne manual injector with sample loop of 20 µL, two LC-20AT pumps, a DUG-20A3 degasser, and an SPD-20 DAD detector were used in this work. The separation was performed in an Agilent Zorbax C-18 column (5.0 µm, 4.6 mm, 250 mm) in reverse-phase (RP) mode using a mobile phase flow rate of 1 mL $min^{-1}$. The gradient was carried out with 65% of ACN (A) and 35% of ultrapure water (B) from 0 to 4 min; then, mobile phase A was increased to 93% from 4 to 5 min, and to 100 % from 5 to 8 min keeping this condition up to 18 min. From 18 to 25 min the concentration of A was kept at 100%. The following cleaning method using a flow rate of 1.5 mL $min^{-1}$ was adopted between runs: from 0 - 5 min using 100% of A; afterwards, from 5 -10 min the concentration of B was increased to 98%. Finally, the initial composition of 65 % of A and 35% of B at 1.0 mL $min^{-1}$ was established. The wavelengths monitored were 250 nm for BZP, 200 nm for MTC, 270 nm for TCC, 245 for PDM and 300 nm for 4-MBC and EHMC.

A semiautomated 96-well plate system, obtained from Brüder Mannesmann Werkzeuge (Remscheid, NRW, Germany), was used to perform the extractions/dilution studies. Neodymium rod magnets (N35, 3 mm x 8.5 mm, 0.595 Tesla) were purchased from Ímã Shop (São Paulo, SP, Brazil).

### *2.3. Synthesis of the cation precursors*

Synthesis of the CPs was carried out according to the procedures previously described [12,24,29]. In order to obtain $[Ni(C_4IM)_4^{2+}]2[Cl^-]$, 4.0 mmol of $NiCl_2$ was reacted with 16 mmol of N-butylimidazole in water, and the reaction was maintained at room temperature overnight. Then, the solvent was removed under reduced pressure and the solid product was dried in a vacuum oven at 60 ºC. Regarding $[Ni(BeIM)_4^{2+}]2[Cl^-]$, 4.0 mmol of $NiCl_2$ was reacted with 16 mmol of N-benzylimidazole and the reaction carried out at 80 ºC [24]. For the $[Co(C_4IM)_4^{2+}]2[Cl^-]$ IL, $CoCl_2$ and N-butylimidazole at molar ratio of 1:4 were used, and the reaction was maintained at 100 ºC for 24 h; then, the product was cooled for 2 h and the solid material was washed with diethyl ether and dried for 24 h in a vacuum oven at 40 ºC [29]. Based on elemental analysis, the composition of these products were consistent with the expected structures [29].

### *2.4. In situ Pa-DDE/MIL-based procedure*

Neodymium rod magnets were adapted in the ends of the 96-well plate blades, as previously reported [19]. 1.25 mL of sample was added in the 96-well plate vials, followed by the addition of 100 µL of an aqueous solution of $[Co(C_4IM)_4^{+2}]2[Cl^-]$ at concentration of 40 g $L^{-1}$. After 5 min of vigorous agitation, 40 µL of an aqueous solution of $LiNTf_2$ (92 g $L^{-1}$) was added into the vials with aid of a multichannel pipette and the agitation was maintained for 75 min. Then, the MIL microdroplets collected in the rod magnets were diluted in 20 µL of ACN and the solution was injected in the HPLC-DAD. This complete procedure is shown in Figure 1.

**Figure 1**

### *2.5. Optimization of the in situ Pa-DDE/MIL based procedure*

Firstly, the extraction efficiency of three different cation precursors $[Co(C_4IM)_4^{+2}]2[Cl^-]$, $[Ni(C_4IM)_4^{+2}]2[Cl^-]$, $[Ni(BeIM)_4^{+2}]2[Cl^-]$ was performed through a univariate planning (n = 3) using

166 5 mg of the MIL ([cation precursor][$NTf_2^-$]) at molar ratio of 1:2, with this ratio being chosen based
167 on previous studies [24]. A full-factorial design was used to examine the influence of the following
168 variables: cation precursor mass (3 – 12 mg), stirring time (15 – 45 min), concentration of NaCl (0 –
169 10% w/v) and pH of the sample (3 – 9), as shown in Table S-1. A Box-Behnken design was then
170 applied to assess the significant parameters of the full factorial design. This last design was used to
171 optimize conditions regarding cation precursor mass (3 – 5 mg), stirring time (45 – 105 min) and
172 concentration of NaCl (0 – 10% w/v). All experiments were performed using 1.25 mL of sample, 125
173 µL of the cation precursor solution and 50 µL of the anion precursor solution, as shown in Table S-
174 2.

175 Finally, evaluation of the addition of a disperser solvent was performed by univariate approach,
176 in triplicate. These experiments were performed with 1.25 mL of ultrapure water spiked with 300 µg
177 $L^{-1}$ of the analytes, using the optimized conditions. Firstly, CP was added, then 60 µL of ACN, MeOH
178 or acetone were added to the spiked sample, and agitation was maintained for 5 min. Afterwards,
179 AER was added and the solution stirred for 75 min. These results were compared with those
180 performed without the addition of dispersive solvent, also performed in triplicate.

181 *2.6. Assessment of the analytical figures of merit and method application*

182 Analytical figures of merit such as linear range, coefficient of determination ($R^2$), limit of
183 detection (LOD), limit of quantification (LOQ), accuracy, precision, enrichment factor, and
184 robustness were determined using the optimized extraction conditions.

185 Calibration curves for each analyte were obtained using tap water samples spiked at five
186 concentrations. The limit of quantification (LOQ) was considered the first concentration of the linear
187 range with adequate precision (≤20 %) and the limit of detection (LOD) was determined as LOQ/3.33.
188 Precision was assessed through intraday assays performed at three concentrations of each analyte
189 (LOQ, 150 and 500 µg $L^{-1}$) in triplicate, and interday precision was performed at 150 µg $L^{-1}$ in

triplicate in three different days (n = 9). The results are represented as relative standard deviation (RSD) of the chromatographic peak areas for each analyte.

The enrichment factor (EF) was determined as the ratio between the response of the extraction using the proposed method performed in an ultrapure water sample spiked with 500 μg $L^{-1}$ ($C_{mil}$), and the response obtained with the direct injection of this spiked sample ($C_0$). The real samples were collected in two different points of a stream (sample A and B) located at the University Campus (Florianópolis, SC, Brazil) and a river located in Rodeio (SC, Brazil). The accuracy was assessed through relative recovery performed in triplicate using three environmental aqueous samples spiked at three concentration levels (LOQ, 150 and 500 μg $L^{-1}$).

Finally, the robustness was performed using the Youden strategy, in which 7 parameters can be evaluated through eight experiments consisting of the combination of small variations of some parameters [30], as shown in Table S-3. The results were evaluated using the geometric means of the chromatographic peak areas of the analytes and presented according to the Lenth's plot. The experiments were performed with ultrapure water spiked with 300 μg $L^{-1}$ of the analytes and the parameters consisted of volume of $[Co(C_4IM)_4^{+2}]2[Cl^-]$ (40 g $L^{-1}$), volume of a $LiNTf_2$ (92 g $L^{-1}$), dispersion time performed between the addition of cation and anion precursors, stirring time, sample volume and ACN volume.

## 3. Results and discussions

### *3.1. Comparison of the extraction efficiency for three cation precursors*

In this study, a previous evaluation of the extraction efficiency of the cation precursors ($[Co(C_4IM)_4^{+2}]2[Cl^-]$, $[Ni(C_4IM)_4^{+2}]2[Cl^-]$ and $[Ni(BeIM)_4^{+2}]2[Cl^-]$) was performed for the development of *in situ* Pa-DDE approach using $[Li^+][NTf_2^-]$ as AER. In this particular case, the extraction efficiency was considered as the average of the normalized chromatographic peak areas of the compounds being studied.

214 The results of this initial comparison were evaluated through ANOVA and included in
215 Supplementary Information (Table S-4). This study indicated that statistically similar results were
216 obtained for the three CPs tested. However, some operational difficulties were observed when using
217 $[Ni(BeIM)_4^{2+}]2[Cl^-]$ due to a lower solubility in water. Thus, $[Co(C_4IM)_4^{2+}]2[Cl^-]$ was selected for
218 the subsequent studies since the analytical response was satisfactory and the physicochemical
219 characteristics of this MIL did not provide operational limitations.

220 *3.2. Full-factorial design*

221 A full-factorial design was performed for evaluating the parameters that can affect the
222 extraction efficiency including mass of CP $[Co(C_4IM)_4^{+2}]2[Cl^-]$ (mg), stirring time, concentration of
223 NaCl (% w/v) and sample pH. The results were evaluated using the geometric means of
224 chromatographic peak areas for the analytes and *Statsoft Statistica 7*® was used for the statistical
225 treatment. A Pareto chart involving the above-mentioned parameters is shown in Figure 2. This chart
226 was obtained considering two-way interactions among the variables with a coefficient of
227 determination ($R^2$) of 0.973, which shows a good correlation between the experimental data and the
228 model proposed.

229 **Figure 2**

230 According to Figure 2, the mass of $[Co(C_4IM)_4^{+2}]2[Cl^-]$ , stirring time, concentration of NaCl
231 and the interactions between mass of CP/stirring time, mass of CP/concentration of NaCl and stirring
232 time/concentration of NaCl were considered significant at a 95% level of confidence ($p<0.05$).
233 Therefore, based on these results, the mass of CP, stirring time and concentration of NaCl were
234 studied in more depth through a Box-Behnken design.

235 Regarding sample pH, the Pareto chart pointed out that this variable was not significant in
236 the extraction performance. This behavior can be associated with the pKa of the analytes (shown in
237 Table S-5) in which most of them did not possess ionizable groups in their chemical structures.
238 Therefore, subsequent experiments were performed without pH adjustments.

239 *3.3. Box-Behnken design for the CP mass, stirring time and concentration of NaCl*

240 A Box-Behnken design was performed to evaluate the significant variables according to the
241 full factorial design described in section 3.2. In this study, stirring time (45 - 105 min), mass of CP
242 (3 - 5 mg) and NaCl concentration (0 - 10 % w/v) were evaluated. Figure 3 shows the response
243 surfaces obtained based on the geometric means of the chromatographic peak areas for the analytes.
244 These surfaces were obtained using a quadratic model considering two-way interactions, with $R^2$ =
245 0.9924 showing good correlation between the data obtained and the statistical model proposed.

246 **Figure 3**

247 According to Figure 3, higher responses were obtained using stirring time and mass of CP
248 close to the central condition (75 min and 4 mg). Even with 75 min of stirring time, the sample
249 throughput was not hindered since with this experimental configuration allows for the simultaneous
250 processing of up to 96 samples. Regarding the concentration of NaCl, this variable exhibited a less
251 pronounced effect on the overall response, which was confirmed by ANOVA in Table S-6. In this
252 study, most of the analytes exhibited low polarity with log P values higher than 3.18 (see Table S-5)
253 and the addition of salt did not significantly affect the solvation properties of the compounds.
254 Therefore, the optimized condition consisted of 4 mg of $[Co(C_4IM)_4^{2+}]2[Cl^-]$ and 75 min of stirring
255 time without addition of NaCl.

256 3.4. *Evaluation of the addition of a disperser solvent*

257 In order to evaluate the extraction efficiency of the methodology with the use of a disperser
258 solvent, experiments were performed using methanol, acetonitrile and acetone as disperser solvents
259 using the optimized procedure. The results were compared with those obtained without the addition
260 of organic solvent. The results are shown as the average of the normalized chromatographic peak
261 areas in Figure S-1. As can be seen in Fig S-1, the use of disperser solvents was not significant in the
262 overall response for the analytes. Since the MIL is prepared by a solvent-free synthesis, only aqueous

263 solutions of the MIL precursors were required in this methodology, and this strategy agreed with the
264 fundamental of the *green* aspects regarding modern analytical methodologies.

265 Also, aiming to verify the method performance without the *in-situ* generation of the MIL,
266 additional experiments were performed using the same hydrophobic MIL previously synthesized
267 (without *in-situ* reaction). However, it was observed that the MIL strongly adhered to the wall of the
268 extraction vials, not being possible to collect or disperse it into the sample since no disperser solvent
269 is used in this methodology.

270

271 *3.5. Assessment of the main analytical figures of merit and method's application*

272 Calibration curves for each analyte were obtained using the optimized procedure and tap water
273 spiked at five concentration levels. Table 1 shows the values obtained for LOD, LOQ, $R^2$, linear range
274 and enrichment factor (EF). LOD and LOQ were 7.5 and 25 μg $L^{-1}$ for all analytes, respectively.
275 Linear ranges were found to vary from 25 to 500 μg $L^{-1}$ with coefficients of determination ($R^2$) higher
276 than 0.989. Enrichment factors of the methodology ranged from 7 (for MTC) to 22 (for TCC).

277 **Table 1.** Analytical parameters of merit for the developed method.

| Analyte | LOD (μg $L^{-1}$) | LOQ (μg $L^{-1}$) | Linear Range (μg $L^{-1}$) | $R^2$ | EF |
|---|---|---|---|---|---|
| BZF | 7.5 | 25 | 25 – 500 | 0.997 | 8 |
| MTC | 7.5 | 25 | 25 – 500 | 0.989 | 7 |
| TCC | 7.5 | 25 | 25 – 500 | 0.991 | 22 |
| PDM | 7.5 | 25 | 25 – 500 | 0.996 | 13 |
| 4-MBC | 7.5 | 25 | 25 – 500 | 0.997 | 14 |
| EHMC | 7.5 | 25 | 25 – 500 | 0.991 | 16 |

278

279 The results obtained for precision and accuracy are shown in Table 2. Intraday precision varied
280 from 6.4 to 20.6% and interday precision varied from 11.6 to 22.9%. The accuracy of the method was

evaluated through relative recovery assays performed in three environmental water samples (A, B and C) and the values ranged from 53.9 to 129.1%. Precision and %RR were considered satisfactory since most of the results are in agreement with the international guidelines [32]. Figure 4 shows a chromatogram obtained from a river water sample spiked with 500 μg $L^{-1}$ of each analyte (A) and from a blank water sample (B). No response was detected for the analytes in the water samples analyzed.

**Table 2.** Precision and accuracy for the developed method.

| Analyte | Concentration (μg $L^{-1}$) | Intraday precision (n = 3) | Interday precision (n = 9) | Relative recovery (%RR) | | |
|---|---|---|---|---|---|---|
| | | | | Sample A | Sample B | Sample C |
| BZF | 25 | 13.5 | 11.6 | 88.5 (± 26.1) | 108.5 (± 26.9) | 55.8 (± 8.3) |
| | 150 | 18.5 | | 77.7 (± 6.5) | 93.7 (± 14.7) | **53.9** (± 4.8) |
| | 500 | 8.8 | | 75.3 (± 11.6) | 103.5 (± 7.7) | 62.0 (± 16.3) |
| MTC | 25 | 15.5 | 14.1 | 88.7 (± 5.3) | 100.8 (± 15.8) | 77.9 (± 7.9) |
| | 150 | 11.7 | | 75.3 (± 2.1) | 95.6 (± 3.5) | 63.0 (± 9.4) |
| | 500 | 16.7 | | 73.3 (± 9.1) | 74.7 (± 12.2) | 67.6 (± 16.1) |
| TCC | 25 | 13.9 | 16.3 | 98.5 (± 22.4) | 96.0 (± 17.7) | 83.0 (± 14.6) |
| | 150 | **6.4** | | 100.0 (± 3.5) | 109.2 (± 17.7) | 85.2 (± 7.8) |
| | 500 | 11.5 | | 101.3 (± 11.6) | 124.2 (± 3.2) | 104.3 (± 2.8) |
| PDM | 25 | 19.2 | 16.7 | 93.2 (± 24.5) | **129.1** (± 2.3) | 118.8 (± 7.4) |
| | 150 | 9.6 | | 102.7 (± 5.4) | 101.7 (± 14.2) | 78.8 (± 6.2) |
| | 500 | 17.7 | | 95.6 (± 12.4) | 126.0 (± 2.5) | 95.0 (± 8.0) |
| 4-MBC | 25 | 13.3 | 19.5 | 84.8 (± 29.5) | 118.8 (± 4.1) | 104.5 (± 4.7) |
| | 150 | 7.9 | | 88.0 (± 5.1) | 100.3 (± 11.1) | 69.6 (± 8.1) |
| | 500 | 15.5 | | 80.7 (± 9.9) | 119.0 (± 0.7) | 79.8 (± 7.9) |
| EHMC | 25 | **20.6** | 22.9 | 102.0 (± 22.9) | 110.8 (± 7.2) | 105.2 (± 7.3) |
| | 150 | 8.7 | | 112.5 (± 4.6) | 98.8 (± 10.2) | 66.6 (± 3.2) |
| | 500 | 13.8 | | 111.7 (± 8.5) | 119.8 (± 2.7) | 84.4 (± 3.0) |

**Figure 4**

Finally, a robustness study was performed in order to evaluate small variations in CP solution volume, AER solution volume, CP dispersion time, stirring time, sample volume and ACN volume. The experiments are listed in Table S-3 and the results are shown in Figure 5. The graph represented

293 in Figure 5 exhibits the margin error (ME) and the simultaneous margin error (SME). When
294 evaluating several effects, SME must be taken into account [30] and none of the parameters studied
295 were considered significant. Therefore, the method can be considered robust.

296 **Figure 5**

297 *3.6. Comparison with data from the literature*

298 A comparison of the main features of the proposed method with others from the literature for
299 the determination of the analytes in water samples is shown in Table 3. The use of alternative solvents
300 such as the deep eutectic solvents (DES) and ILs in sample preparation techniques has been an
301 important substitution in the place of traditional organic solvents [14,19,22,33–36]. Despite being
302 considered green solvents, some of the methods cited in Table 3 still use toxic organic solvents for
303 the synthesis and production of such alternative solvents [14,19,22]. This method emerges as a green
304 alternative as it exhibits the important advantages of using MILs that were synthesized in aqueous
305 media and the use of only 20 µL of ACN for the dilution step.

306 Although LODs were slightly higher than those reported in other studies, this method exhibits
307 high-throughput since the extraction time per sample is 0.78 min in comparison to other methods that
308 needed more than 30 min per sample [35–37]. In addition, the Pa-DDE approach proposed in this
309 study follows with the principles of GAC regarding low sample consumption, since a volume of only
310 1.25 mL was necessary.

311 One of the advantages of the *in situ* formation of MILs is related the metathesis reaction in
312 which microdroplets of the MIL are formed in the sample solution, thereby increasing the surface
313 area of the MIL and providing higher extraction efficiencies [25]. This was previously demonstrated
314 by comparing the extraction efficiency using DLLME performed through the conventional and *in situ*
315 approaches. Superior results were obtained for all of the analytes using the *in situ* approach [24].

316 Another advantage of the *in situ* formation of the MIL consists of avoiding operational issues related
317 to the pipetting of MILs due to their high viscosity [25].

318 **Table 3.** Analytical features of the proposed methodology compared to previously reported studies.

| Sample preparation | Separation/ Detection | Analytes | Extraction solvent | Sample volume (mL) | LOD (μg $L^{-1}$) | Extraction time (min/per sample) | Ref. |
|---|---|---|---|---|---|---|---|
| *In situ* Pa-DDE/MIL | HPLC-DAD | BZF. MTC, TCC, PDM, 4-MBC, EHMC | $[Co(C_4IM)_4^{+2}]2[NTf_2^-]$ | 1.25 | 7.5[a, b, c, d, e, f] | 0.78 | This work |
| Pa-SDME/MIL | HPLC-DAD | BZF, TCC | $[P_{6,6,6,14}^+]_2[MnCl_4^{-2}]$ | 1.5 | 1.5[a] and 3.0[c] | 0.94 | [19] |
| TC-IL-DLPME | HPLC-UV | BZF | [HMIM][FAP] | 10 | 0.3[a] | 20 | [33] |
| AA-LLME-SFDES | -HPLC-UV | BZF | DES $C_{10}$:$C_{12}$ (2:1) | 5 | 0.45[a] | > 3 | [34] |
| SBDLME | TD-GC-MS | EHMC, 4-MBC | $[P_{6,6,6,14}][Ni(hfacac)_3]$ | 25 | 0.152[e] and 0.153[f] | 10 | [22] |
| HF-DLLME | HPLC-DAD | 4-MBC, TCC | Octanol and hexane | 20 | 3.0[c, e] | ~ 60 | [37] |
| IL-SDME | LC-UV | 4-MBC, EHMC | $[C_6MIM][PF_6]$ | 20 | 0.06[e] and 0.19[f] | 37 | [35] |
| IL-HF-LPME | HPLC-UV | BZF, 4-MBC | [HMIM][FAP] | 10 | 0.2[a] and 0.3[e] | 50 | [36] |
| DLLME/MIL | HPLC-DAD | TCC, MTC | $[P_{6,6,6,14}]_2[MnCl_4]$ | 3 | 1.5[b, c] | 5 | [14] |
| VA-DLLME | GC-MS/MS | PDM | $CHCl_3$ | 7.5 | NF | 5 | [38] |

319 [a] BZF, [b] MTC, [c] TCC, [d] PDM, [e] 4-MBC, [f] EHMC.

320 TC-IL-DLPME: temperature controlled ionic liquid dispersive liquid phase microextraction; [HMIM][FAP]: 1- hexyl-3-methylimidazolium
321 tris(pentafluoroethyl)trifluorophosphate; AA-LLME-SFDES: Air assisted liquid-liquid microextraction based on solidification of floating deep
322 eutectic solvent; SBDLME: Stir bar dispersive liquid microextraction; TD-GC-MS: termal desorption gas chromatography coupled with
323 detection mass spectroscopy; HF-DLLME: Hollow fiber-supported dispersive liquid-liquid microextraction; $[C_6MIM][PF_6]$: 1-hexyl-3-
324 methylimidazolium hexafluorophosphate; IL-HF-LPME: Ionic liquid based hollow fiber supported liquid phase microextraction; VA-DLLME:
325 Vortex assisted dispersive liquid-liquid microextraction; NF: data not found

## 4. Conclusions

An analytical methodology based on *in situ* formation of MILs combined with the 96-well plate was successfully developed for the first time. The developed *in situ* Pa-DDE method was optimized and exhibited satisfactory analytical performance. This configuration embodied the high-throughput analysis of a 96-well plate system and green aspects related to the *in situ* formation of the MILs. In addition, the approach requires low consumption of organic solvent and sample. This study consists of a greener and eco-friendly alternative to previously proposed methods by our research group since the synthesis of the MILs does not require organochlorine solvents. On the other hand, some issues related to the MIL solubility in aqueous samples were also observed, and strategies to overcome this based on structural tuning of the MIL can be further studied and exploited.

## CRediT authorship contribution statement

**Camila Will**: Methodology, Validation, Investigation, Writing - original draft. **Ricardo Dagnoni Huelsmann**: Methodology, Validation, Investigation, Writing - review & editing. **Gabriela Mafra**: Conceptualization, Writing - review & editing. **Josias Merib**: Conceptualization, Writing - review & editing. **Jared L. Anderson**: Writing - review & editing, Resources, Funding acquisition. **Eduardo Carasek**: Conceptualization, Writing - review & editing, Project administration, Funding acquisition, Supervision.

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## References

[1] A. Gałuszka, Z. Migaszewski, J. Namieśnik, The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices, TrAC Trends Anal. Chem. 50 (2013) 78–84. https://doi.org/10.1016/j.trac.2013.04.010.

[2] P.T. Anastas, Green Chemistry and the Role of Analytical Methodology Development, Crit. Rev. Anal. Chem. 29 (1999) 167–175. https://doi.org/10.1080/10408349891199356.

[3] D.C. Morelli, G. Mafra, A.V. Santos, J. Merib, E. Carasek, Designing a green device to BAμE: Recycled cork pellet as extraction phase for the determination of parabens in river water samples, Talanta. 219 (2020) 121369. https://doi.org/10.1016/j.talanta.2020.121369.

[4] E. Carasek, L. Morés, J. Merib, Basic principles, recent trends and future directions of microextraction techniques for the analysis of aqueous environmental samples, Trends Environ. Anal. Chem. 19 (2018) e00060. https://doi.org/10.1016/j.teac.2018.e00060.

[5] E. Carasek, J. Merib, G. Mafra, D. Spudeit, A recent overview of the application of liquid-phase microextraction to the determination of organic micro-pollutants, TrAC Trends Anal. Chem. 108 (2018) 203–209. https://doi.org/10.1016/j.trac.2018.09.002.

[6] M. Koel, Do we need Green Analytical Chemistry?, Green Chem. 18 (2016) 923–931. https://doi.org/10.1039/C5GC02156A.

[7] E. Carasek, G. Bernardi, S.N. do Carmo, C.M.S. Vieira, Alternative Green Extraction Phases Applied to Microextraction Techniques for Organic Compound Determination, Separations. 6 (2019) 35. https://doi.org/10.3390/separations6030035.

[8] R. Marcinkowska, K. Konieczna, Ł. Marcinkowski, J. Namieśnik, A. Kloskowski, Application of ionic liquids in microextraction techniques: Current trends and future perspectives, TrAC Trends Anal. Chem. 119 (2019) 115614. https://doi.org/10.1016/j.trac.2019.07.025.

376 [9] K.D. Clark, M.N. Emaus, M. Varona, A.N. Bowers, J.L. Anderson, Ionic liquids: solvents and
377 sorbents in sample preparation, J. Sep. Sci. 41 (2018) 209–235.
378 https://doi.org/10.1002/jssc.201700864.
379 [10] M. Sajid, Magnetic ionic liquids in analytical sample preparation: A literature review, TrAC
380 Trends Anal. Chem. 113 (2019) 210–223. https://doi.org/10.1016/j.trac.2019.02.007.
381 [11] K.D. Clark, O. Nacham, J.A. Purslow, S.A. Pierson, J.L. Anderson, Magnetic ionic liquids in
382 analytical chemistry: A review, Anal. Chim. Acta. 934 (2016) 9–21.
383 https://doi.org/10.1016/j.aca.2016.06.011.
384 [12] M.J. Trujillo-Rodríguez, J.L. Anderson, In situ generation of hydrophobic magnetic ionic
385 liquids in stir bar dispersive liquid-liquid microextraction coupled with headspace gas
386 chromatography, Talanta. 196 (2019) 420–428. https://doi.org/10.1016/j.talanta.2018.12.071.
387 [13] J. Merib, D.A. Spudeit, G. Corazza, E. Carasek, J.L. Anderson, Magnetic ionic liquids as
388 versatile extraction phases for the rapid determination of estrogens in human urine by
389 dispersive liquid-liquid microextraction coupled with high-performance liquid
390 chromatography-diode array detection, Anal. Bioanal. Chem. 410 (2018) 4689–4699.
391 https://doi.org/10.1007/s00216-017-0823-7.
392 [14] A.C. da Silva, G. Mafra, D. Spudeit, J. Merib, E. Carasek, Magnetic ionic liquids as an
393 efficient tool for the multiresidue screening of organic contaminants in river water samples,
394 Sep. Sci. PLUS. 2 (2019) 51–58. https://doi.org/10.1002/sscp.201900010.
395 [15] D. Cao, X. Xu, S. Xue, X. Feng, L. Zhang, An in situ derivatization combined with magnetic
396 ionic liquid-based fast dispersive liquid-liquid microextraction for determination of biogenic
397 amines in food samples, Talanta. 199 (2019) 212–219.
398 https://doi.org/10.1016/j.talanta.2019.02.065.
399 [16] X. Feng, X. Xu, Z. Liu, S. Xue, L. Zhang, Novel functionalized magnetic ionic liquid green
400 separation technology coupled with high performance liquid chromatography: A rapid

401 approach for determination of estrogens in milk and cosmetics, Talanta. 209 (2020) 120542.
402 https://doi.org/10.1016/j.talanta.2019.120542.
403 [17] M.N. Emaus, J.L. Anderson, Allelic discrimination between circulating tumor DNA fragments
404 enabled by a multiplex-qPCR assay containing DNA-enriched magnetic ionic liquids, Anal.
405 Chim. Acta. (2020). https://doi.org/10.1016/j.aca.2020.04.078.
406 [18] E.F. Fiorentini, B.V. Canizo, R.G. Wuilloud, Determination of As in honey samples by
407 magnetic ionic liquid-based dispersive liquid-liquid microextraction and electrothermal atomic
408 absorption spectrometry, Talanta. 198 (2019) 146–153.
409 https://doi.org/10.1016/j.talanta.2019.01.091.
410 [19] G. Mafra, A.A. Vieira, J. Merib, J.L. Anderson, E. Carasek, Single drop microextraction in a
411 96-well plate format: A step toward automated and high-throughput analysis, Anal. Chim.
412 Acta. 1063 (2019) 159–166. https://doi.org/10.1016/j.aca.2019.02.013.
413 [20] E. Fernández, L. Vidal, A. Canals, Hydrophilic magnetic ionic liquid for magnetic headspace
414 single-drop microextraction of chlorobenzenes prior to thermal desorption-gas
415 chromatography-mass spectrometry, Anal. Bioanal. Chem. 410 (2018) 4679–4687.
416 https://doi.org/10.1007/s00216-017-0755-2.
417 [21] M.J. Trujillo-Rodríguez, V. Pino, J.L. Anderson, Magnetic ionic liquids as extraction solvents
418 in vacuum headspace single-drop microextraction, Talanta. 172 (2017) 86–94.
419 https://doi.org/10.1016/j.talanta.2017.05.021.
420 [22] A. Chisvert, J.L. Benedé, J.L. Anderson, S.A. Pierson, A. Salvador, Introducing a new and
421 rapid microextraction approach based on magnetic ionic liquids: Stir bar dispersive liquid
422 microextraction, Anal. Chim. Acta. 983 (2017) 130–140.
423 https://doi.org/10.1016/j.aca.2017.06.024.

[23] M. Baghdadi, F. Shemirani, In situ solvent formation microextraction based on ionic liquids: A novel sample preparation technique for determination of inorganic species in saline solutions, Anal. Chim. Acta. 634 (2009) 186–191. https://doi.org/10.1016/j.aca.2008.12.017.

[24] M.J. Trujillo-Rodríguez, J.L. Anderson, In situ formation of hydrophobic magnetic ionic liquids for dispersive liquid-liquid microextraction, J. Chromatogr. A. 1588 (2019) 8–16. https://doi.org/10.1016/j.chroma.2018.12.032.

[25] A.N. Bowers, M.J. Trujillo-Rodríguez, M.Q. Farooq, J.L. Anderson, Extraction of DNA with magnetic ionic liquids using in situ dispersive liquid–liquid microextraction, Anal. Bioanal. Chem. 411 (2019) 7375–7385. https://doi.org/10.1007/s00216-019-02163-9.

[26] J.P. Hutchinson, L. Setkova, J. Pawliszyn, Automation of solid-phase microextraction on a 96-well plate format, J. Chromatogr. A. 1149 (2007) 127–137. https://doi.org/10.1016/j.chroma.2007.02.117.

[27] M. Alexovič, Y. Dotsikas, P. Bober, J. Sabo, Achievements in robotic automation of solvent extraction and related approaches for bioanalysis of pharmaceuticals, J. Chromatogr. B. 1092 (2018) 402–421. https://doi.org/10.1016/j.jchromb.2018.06.037.

[28] D. Lopes, A.N. Dias, J. Merib, E. Carasek, Hollow-fiber renewal liquid membrane extraction coupled with 96-well plate system as innovative high-throughput configuration for the determination of endocrine disrupting compounds by high-performance liquid chromatography-fluorescence and diode array detection, Anal. Chim. Acta. 1040 (2018) 33–40. https://doi.org/10.1016/j.aca.2018.07.032.

[29] D. Chand, M.Q. Farooq, A.K. Pathak, J. Li, E.A. Smith, J.L. Anderson, Magnetic ionic liquids based on transition metal complexes with N-alkylimidazole ligands, New J. Chem. 43 (2018) 20–23. https://doi.org/10.1039/C8NJ05176C.

[30] F. Leonardi, M. Veschetti, S. Tonnarini, F. Cardellini, R. Trevisi, A step towards accreditation: A robustness test of etching process, Appl. Radiat. Isot. 102 (2015) 93–97. https://doi.org/10.1016/j.apradiso.2015.05.002.

[31] M.J. Trujillo-Rodríguez, P. Rocío-Bautista, V. Pino, A.M. Afonso, Ionic liquids in dispersive liquid-liquid microextraction, TrAC Trends Anal. Chem. 51 (2013) 87–106. https://doi.org/10.1016/j.trac.2013.06.008.

[32] M. Rambla-Alegre, J. Esteve-Romero, S. Carda-Broch, Is it really necessary to validate an analytical method or not? That is the question, J. Chromatogr. A. 1232 (2012) 101–109. https://doi.org/10.1016/j.chroma.2011.10.050.

[33] Y. Zhang, H.K. Lee, Determination of ultraviolet filters in environmental water samples by temperature-controlled ionic liquid dispersive liquid-phase microextraction, J. Chromatogr. A. 1271 (2013) 56–61. https://doi.org/10.1016/j.chroma.2012.11.047.

[34] K. Zhang, S. Li, Y. Wang, J. Fan, G. Zhu, Air-assisted liquid-liquid microextraction based on solidification of floating deep eutectic solvent for the analysis of ultraviolet filters in water samples by high performance liquid chromatography with the aid of response surface methodology, J. Chromatogr. A. 1618 (2020) 460876. https://doi.org/10.1016/j.chroma.2020.460876.

[35] L. Vidal, A. Chisvert, A. Canals, A. Salvador, Ionic liquid-based single-drop microextraction followed by liquid chromatography-ultraviolet spectrophotometry detection to determine typical UV filters in surface water samples, Talanta. 81 (2010) 549–555. https://doi.org/10.1016/j.talanta.2009.12.042.

[36] D. Ge, H.K. Lee, Ionic liquid based hollow fiber supported liquid phase microextraction of ultraviolet filters, J. Chromatogr. A. 1229 (2012) 1–5. https://doi.org/10.1016/j.chroma.2011.12.110.

[37] D. Lopes, A.N. Dias, V. Simão, E. Carasek, Determination of emerging contaminants in aqueous matrices with hollow fiber-supported dispersive liquid-liquid microextraction (HF-DLLME) and separation/detection by liquid chromatography – Diode array detection, Microchem. J. 130 (2017) 371–376. https://doi.org/10.1016/j.microc.2016.10.011.

[38] O.I. Abdallah, Simultaneous determination of nine dinitroaniline herbicides in environmental samples using a validated vortex-assisted dispersive liquid–liquid microextraction procedure coupled with GC–MS/MS, Chem. Pap. 74 (2020) 2311–2326. https://doi.org/10.1007/s11696-020-01075-8.

**Figure captions**

**Figure 1.** Scheme for *in situ* Pa-DDE/MIL-based procedure.

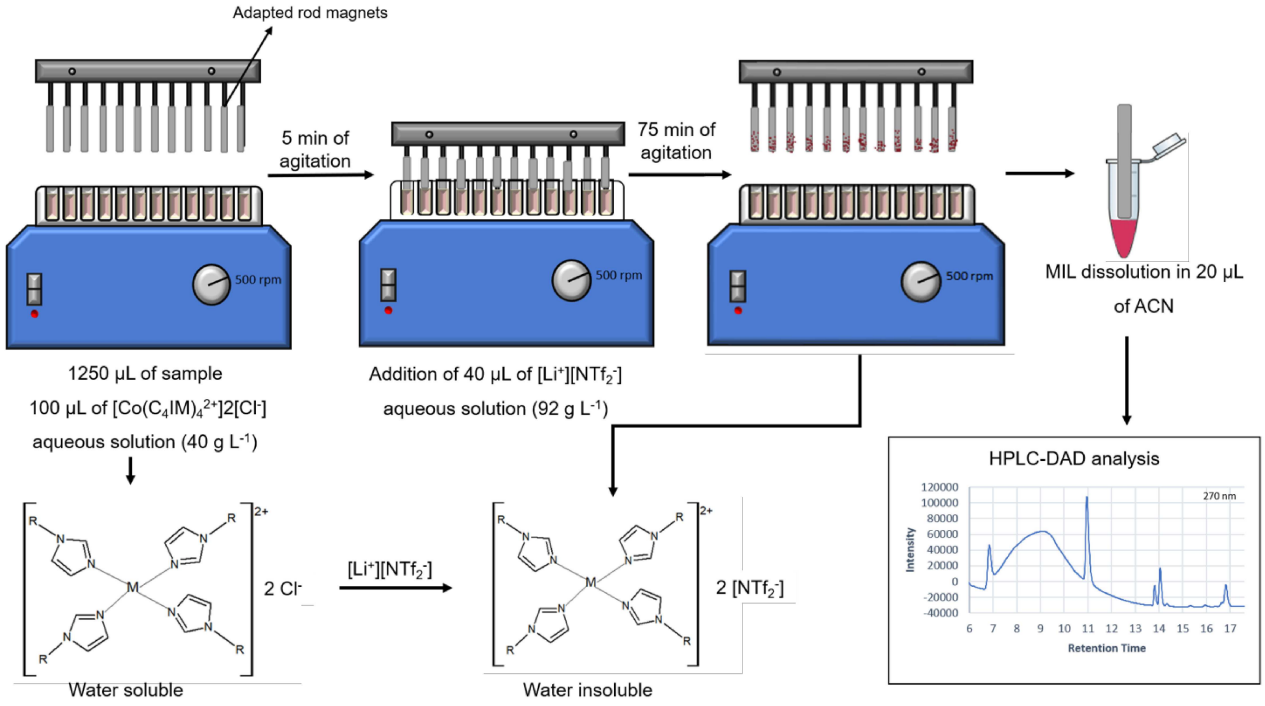
**Figure 2.** Pareto chart obtained for the variables $[Co(C_4IM)_4]Cl_2$ mass, stirring time, % NaCl (w/v) and sample pH.

**Figure 3.** Response surfaces obtained from a Box-Behnken design for the evaluation of $[Co(C_4IM)_4]2Cl$ mass, stirring time and % NaCl (w/v).

**Figure 4.** Chromatograms obtained at 270 nm and 200 nm of extractions from a spiked river water sample with 500 μg $L^{-1}$ of the analytes (A) and blank water sample (B).

**Figure 5.** Lenth's plot for the evaluation of method robustness performed through Youden strategy. (A: $[Co(C_4IM)_4]2Cl$ volume, B: $[Li^+][NTf_2^-]$ volume, C: CP dispersion time, D: stirring time, E: sample volume and F: ACN volume.

## Figure 1




*M= Ni or Co and R= butyl or benzyl

## Figure 2

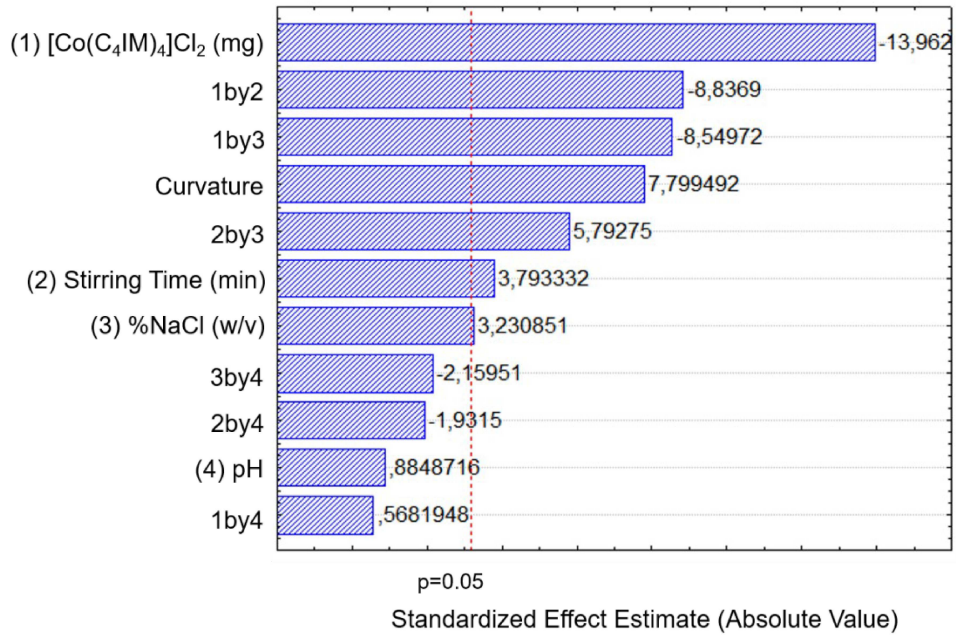


**Figure 3**

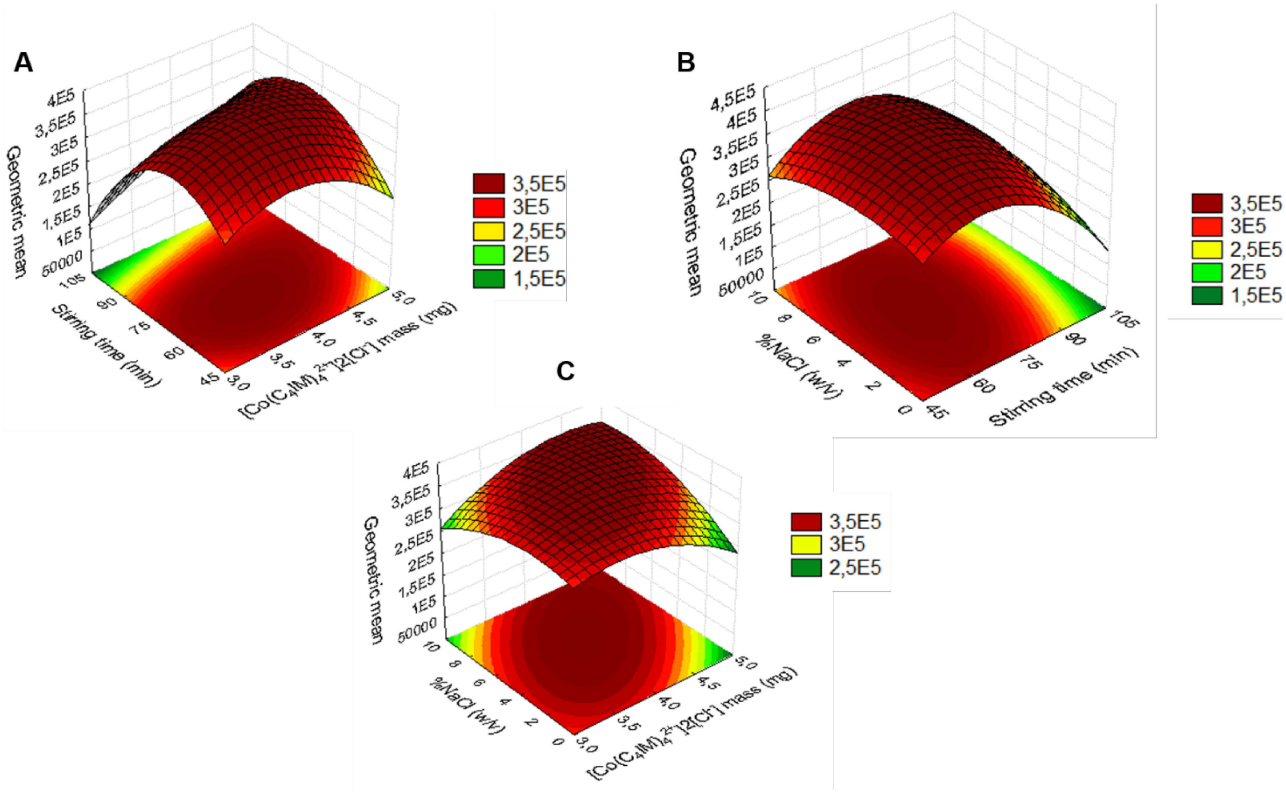
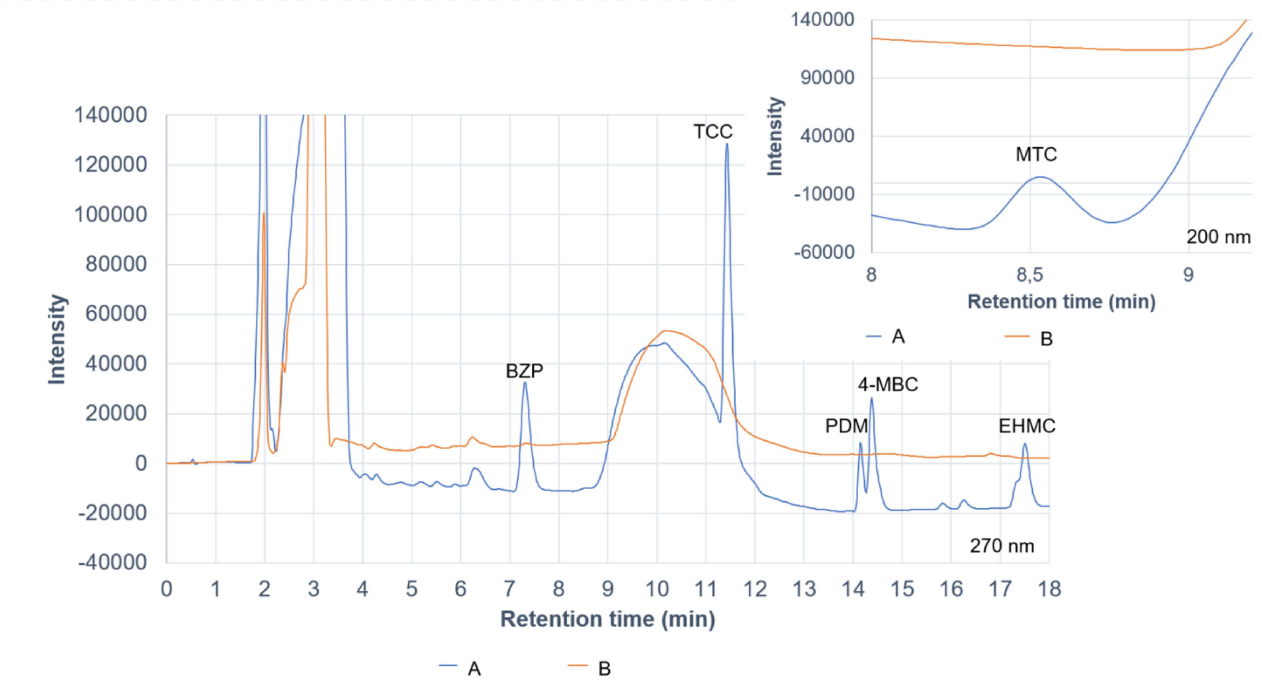



**Figure 4**



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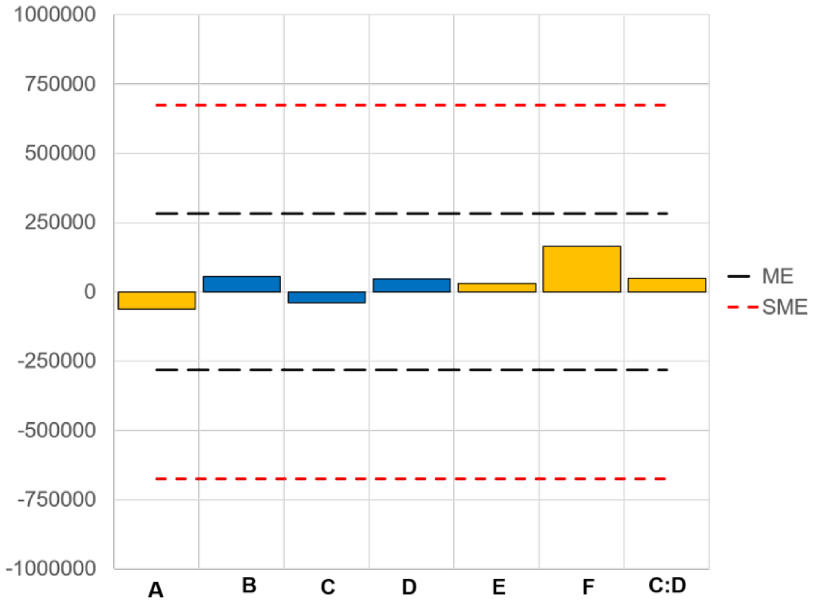
513 **Figure 5**

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