HYPERCROSSLINKED PARTICLES TO EXTRACT SWEETENERS USING DISPERSIVE SOLID-PHASE EXTRCTION FROM ENVIRONMENTAL SAMPLES

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RUNNING TITLE: HYPERCROSSLINKED PARTICLES TO EXTRACT SWEETENERS

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List of non-standard abbreviations used in the text:

ACE – acesulfame; ALI – alitame; ASP – aspartame; BPO – benzoyl peroxide; CE: collision energy; CV: cone voltage; CYC – cyclamate; DCE – 1,2-dichloroethane; d-SPE: dispersive solid-phase extraction; DVB – divinylbenzene; GLY - glycyrrhizic acid; ILODs – instrumental limits of detection; ILOQs – instrumental limits of quantification; ME – matrix effect; MPs – magnetic particles; MRM – multiple reaction monitoring; NHDC – neohesperidin dihydrochalcone; NEO – neotame; OA – oleic acid; PVA – polyvinyl alcohol; R_{app} – apparent recovery; SAC- saccharin; STV – stevioside; SUC – sucralose; SWs – sweeteners; WWTP – waste water treatment plant.

Keywords:

Dispersive solid-phase extraction; environmental water samples; hypercrosslinked magnetic particles; liquid chromatography-tandem mass spectrometry; sweeteners.

ABSTRACT

This work presents a new extraction material, namely Q-100, based on hypercrosslinked magnetic particles that was tested in dispersive solid-phase extraction for a group of sweeteners from environmental samples. The hypercrosslinked Q-100 MPs had the advantage of suitable pore size distribution and high surface area, and showed good retention behaviour towards sweeteners.

Different dispersive solid-phase extraction parameters such as amount of magnetic particles or extraction time were optimized. Under optimum conditions, Q-100 showed suitable apparent recovery, ranging in the case of river water sample from 21% to 88% for all the sweeteners, except for alitame (12%). The validated method based on dispersive solid-phase extraction using Q-100 followed by LC-MS/MS provided good linearity and limits of quantification between 0.01 and 0.1 μg L^{-1}.

The method was applied to analyze samples from river water and effluent wastewater and four sweeteners (acesulfame, saccharin, cyclamate and sucralose) were found in both types of samples.

1. INTRODUCTION

Currently, the consumption of synthetic sweeteners (SWs) is increasing because of their low calorific value, high potency and non-nutritive properties to help to prevent body weight gain, dental cavities and diabetes [1]. They are the largest class of additives in the food industry and are purposely added to food, beverages, personal care products and so on to provide a sweet flavour or as a preservative. In the 1950s, the first generation of SWs was introduced, including aspartame (ASP), saccharin (SAC) and cyclamate (CYC). Subsequently, the second generation of SWs included acesulfame (ACE), sucralose (SUC), neotame (NEO) and alitame (ALI) [2]. In the last decade, ACE, ASP, CYC, SAC, SUC, neohesperidin-dihydrochalcone (NHDC) have been permitted by the European Union (EU) for use in foodstuffs [3]. Moreover, consumption has also increased in the case of SWs of natural origin, such as stevioside (STV) and glycyrrhizic acid (GLY) [1]. Since December 2011, the EU has permitted the use of steviol glycosides in foodstuffs [4]. Due to high consumption, the occurrence of SWs in the aquatic environment has already been demonstrated in previous studies and they are therefore considered as emerging organic contaminants (EOCs) [5-7]. The major source of discharging EOCs in environment water is wastewater treatment plants (WWTPs). Some studies [7,8] have demonstrated that SWs are inadequately removed from WWTPs. As such, they remain in environment and their presence affects the physiology and locomotion behaviour of aquatic species [9], though they are considered nontoxic to humans within regulated concentrations [10, 11]. Therefore, recent research has focused on studying the environmental occurrence, fate and ecotoxicological effect of SWs [8, 9, 11].

To date, a large number of papers about the determination of SWs in environmental water have been published. Most of these are based on solid-phase extraction (SPE), either in off-line [6, 12] or on-line [13] mode, followed by liquid chromatography (LC). Another SPE mode is the dispersive (d-SPE) one, which has been already used for extracting target compounds by dispersing a few mg of sorbent into liquid samples [14]. d-SPE has also been used to clean up extracts from QuEChERS extraction [15].

As well as the most commonly applied silica- [16] and polymeric-based sorbents [17], magnetic particles (MPs) have been also applied in d-SPE, in which they are dispersed in aqueous samples, shortening the equilibrium time and being easily removed from aqueous sample by applying a magnetic field rather than centrifugation or filtration [18]. Numerous MPs have been developed through the incorporation of different functional groups (silica, carbon, surfactants and polymers), which are used in different analytical applications [18, 19], which include the extraction of compounds such as endocrine disruptors [20], drugs [21] and food additives including some SWs [22]. In fact, this is the only study [22] dealing with the extraction of SWs, but from red wine samples.

Recently, a novel Q-100 hypercrosslinked MPs was developed and demonstrated efficient removal of antibiotics from water from a WWTP [23]. An important feature of the Q-100 material are the hypercrosslinked structure, which leads to suitable pore size distribution and high surface area. Because of these properties, hypercrosslinked materials have been fulfilled a broad range of applications [24], including the retention of compounds in sorptive extraction techniques. Specifically, the aim of this study was to evaluate the retention behaviour of Q-100 as material for

113 extraction of a broad group of SWs from environmental water samples using the d-
114 SPE technique followed by LC-tandem mass spectrometry (LC-MS/MS).

115 2. MATERIALS AND METHODS

116 2.1. Reagents and Standards

117 Analytical reagent grade ferrous ferric oxide (Fe_3O_4), ferric chloride hexahydrate
118 ($FeCl_3$, $6H_2O$), ferrous chloride tetrahydrate ($FeCl_2$, $4H_2O$), aqueous ammonia (28
119 wt%), benzoyl peroxide (BPO), toluene, 1,2-dichloroethane (DCE), oleic acid (OA),
120 acetone and methanol (MeOH) were purchased from Shanghai Chemical Reagent
121 Corp. (China). Polyvinyl alcohol (PVA) and divinylbenzene (DVB, 80 wt%) were
122 purchased from Sigma-Aldrich and J&K Chemical Co. Ltd. (Shanghai, China).

123 HPLC grade acetonitrile (ACN) and MeOH were supplied by Scharlab
124 (Barcelona, Spain). Hydrochloric acid (HCl) and formic acid (HCOOH) 95% used to
125 adjust the pH of the sample and mobile phase were purchased from Merck
126 (Darmstadt, Germany). Ammonium hydroxide (NH_4OH) was purchased from Sigma-
127 Aldrich (Steinheim, Germany). Ultrapure water was obtained using an ultrapure water
128 purification system Veolia Waters (Sant Cugat del Vallès, Spain).

129 The individual SW standards were supplied by Sigma-Aldrich and they were:
130 ACE, SAC, CYC, ASP, SUC, ALI, NHDC, STV, NEO and GLY. All the above standards
131 were 99% purity except GLY, which was 70% purity. The chemical structures and
132 properties of all SWs are described in Fig. 1S (supplementary information).

133 Stock solutions of individual standards were prepared by dissolving of pure
134 compound in MeOH at a concentration of 1,000 mg/L, and then stored at -20^{o}C in
135 amber glass bottles. Mixed standard solutions at a concentration of 50 mg/L were

prepared every month by dilution of stock solutions in MeOH and stored at 4ºC. Mixed standard working solutions were prepared daily from mixed standard solutions by appropriate dilution with water:MeOH (9:1, v/v).

2.2. Preparation of hypercrosslinked Q-100 magnetic particles

The hypercrosslinked MPs Q-100 were prepared through the copolymerization reaction reported in a previous work [23]. Briefly, the Fe_3O_4 nanoparticles were prepared through coprecipitation reaction, and coated by OA under a nitrogen atmosphere to enhance the lipophilicity of Fe_3O_4 nanoparticles. The oil phase containing the monomer DVB, the initiator BPO, the porogen toluene and the magnetic core, OA-Fe_3O_4, was stirred at 80ºC in the aqueous phase, which consisted of PVA and sodium sulphate dissolved in distilled water. Afterwards, the obtained MPs were dried and hypercrosslinked in 1,2-DCE at 90ºC for 18 h using anhydrous ferric chloride as catalyst. The obtained Q-100 particles were rinsed and dried. A full characterization data can be found in Fig 2S to 4S. A surface area of ~1,150 m^2/g should be highlighted.

2.3. Dispersive solid phase extraction

For the d-SPE procedure, 100 mg of Q-100 MPs were introduced into a glass vial, and the sample volume (50 mL for river waters and 25 mL for wastewater samples) adjusted to pH 2 with HCl was added to the glass vials. The solution was stirred for 30 min at 900 rpm aided by a magnetic stirrer. After 30 min, the MPs were separated out from the water sample using the filtration assembly and then dried under vacuum for 15 min. Finally, the retained analytes were eluted from the MPs by passing 5 mL of MeOH and 5 mL of 2% NH_4OH in MeOH solvent through the same assembly. The elution solvent was evaporated using a Genevac miVac Duo system concentrator

(Ipswich, United Kingdom) and the dried residue was resuspended with 1 mL of water:MeOH (9:1 v/v) prior to injecting into LC-MS/MS.

To avoid carryover, the Q-100 MPs were cleaned after each use by twice passing 5 mL of MeOH and 5 mL of 2% NH_4OH in MeOH solvent through the same assembly, and then they were vacuum dried.

2.4. Liquid chromatography-tandem mass spectrometry analysis

A 1200 series HPLC system from Agilent Technologies (Waldbronn, Germany) equipped with a degasser, an automatic injector, a column oven and a ultra violet (UV) detector was used during the optimization of the d-SPE. The column used was a Zorbax Eclipse XDB-C_8 (150 mm × 4.6 mm i.d., 5 µm) from Agilent. The column temperature was maintained at 25ºC. The mobile phase flow-rate was set 0.6 mL/min and the sample volume injected was 50 µL. The mobile phase consisted of ultrapure water adjusted to pH 2.5 with HCl and ACN. The gradient started at 5% ACN, which was increased to 40% ACN in 13 min, then to 100% in 11 min and kept constant for 3 min. Then, it was returned to the initial conditions in 2 min, which was held for 8 min to equilibrate the column for further analysis. All the compounds were eluted in 20 min and the total run time was 29 min. All the compounds were detected at 210 nm, except ACE (227 nm) and GLY (250 nm).

Once the d-SPE was optimized, the method was validated with an Agilent 1200 series LC coupled to a 6410 series triple quadrupole mass spectrometer with an electrospray ionization (ESI) interface from Agilent Technologies. The chromatographic conditions used in the LC-MS/MS instrument were the same as for the LC-UV, except that the aqueous mobile phase was adjusted to pH 3 with HCOOH. The analyses were performed in multiple reaction monitoring (MRM) and negative

ionization mode. The operating optimized ESI parameters were as follows: N_2 flow rate 12 L/min, capillary voltage of 4,000 V, nebulizer pressure of 45 psi (N_2) and source temperature of 350ºC. Three MRM transitions (one as a quantifier, two as qualifiers) were selected for each analyte. Just in the case of CYC and SUC, two MRM transitions were monitored due to their poor fragmentation. All information is summarized in Table 1S.

Under the LC-MS/MS conditions, the 10 SWs showed good linearity (determination coefficients R^2>0.9993) in the range of 0.2-50 µg L^{-1} for ACE, SUC, ALI, STV, and GLY, and 0.5-10 µg L^{-1} for SAC, CYC, ASP, NEO, and NHDC. The instrumental limits of detections (ILODs) were evaluated as a signal-to-noise ratio (S/N) of 3:1 and ranged from 0.05-0.5 µg L^{-1}.The lowest points of the calibration curve were taken as the instrumental limits of quantifications (ILOQs).

2.5. Sampling

The surface water samples from the River Ebro were collected from three different locations and the wastewater samples were collected from the influent and effluent wastewater treatment plants (WWTPs) in Tarragona and Reus cities (Spain). All samples were filtered using 1.2 µm glass fibre filters followed by 0.45 µm nylon filters, both from Fisher (Loughborough, UK). The samples were then stored at 4ºC until analysis.

3. RESULTS AND DISCUSSION

3.1. Optimization of d-SPE procedure

For the d-SPE procedure, several parameters were optimized including extraction time, sample pH and volume, the amount of Q-100 MPs, elution solvent and

its volume. These parameters were optimized by LC-UV (conditions described in Section 2.4), and this did not include the compounds CYC and SUC because they do not absorb in the UV range due to the lack of a chromophore group. Initial experimental extraction conditions were: 10 mL of ultrapure water adjusted to pH 3 with HCOOH spiked at 5 µg L^{-1} with the mixture of analytes placed in a ~10 mL glass vial. Then, 50 mg of Q-100 MPs were transferred into a vial and the solution was stirred at 900 rpm, aided by a Teflon-coated magnetic stir bar for 30 min at room temperature for the sorption of the analytes. After sorption, the Q-100 MPs were separated from the sample using the filtration assembly instead of applying an external magnetic field, since Q-100 particles' own limited magnetization as shown in the magnetization curve (Fig. 4S). For the elution of the retained analytes, 3x5 mL of MeOH were passed through the particles in the filtration assembly. The three collected fractions were evaporated to dryness and the residue re-dissolved in water:MeOH (9:1) mixture before injection into the LC system.

3.1.1. Extraction conditions

The recoveries at sample pH 2, 3 and 7 were compared in order to evaluate the effect of pH on extraction recoveries of the SWs, since they possess different physicochemical characteristics (see Fig. S1 for details). As Fig. 1 shows, comparing pH 3 and 7, the recoveries were only 10% to 15% lower at pH 7 than at pH 3. In contrast, when decreasing the pH from 3 to 2, the compounds ACE, SAC, ASP, ALI and GLY presented higher extraction recoveries at pH 2, while no significant decrease was observed for the rest of the compounds. Therefore, considering these results, pH 2 was chosen for further experiments.

To investigate the sample volume, 50 and 100 mL of ultrapure water spiked with mixture of SWs were tested, and it was observed that recoveries decreased (by 10% to 20%) for all analytes when the sample volume increased from 10 mL to 50 mL, although the sensitivity of the method increases. It was also observed that from 50 mL to 100 mL of sample, the recoveries further decreased from 5% to 15% for all compounds. Therefore, 50 mL of sample was selected as a compromise between the sensitivity and recoveries of the method.

The amount of MPs was tested at 50 mg, 100 mg and 150 mg. When the amount of MPs increased from 50 mg to 100 mg, the extraction recoveries increased from 5% to 35% for all compounds. However, when increasing from 100 mg to 150 mg, the MPs formed agglomerates instead of being dispersed in the sample, and the recoveries did not increase more than 10%. Thus, 100 mg of MPs was selected for further experiments.

In order to improve the extraction recovery a longer extraction time (60 min) was tested. However, the results showed that, in the case of 60 min extraction, recoveries of analytes did not increase more than 10% compared to 30 min. In addition, for routine analysis, an extended extraction time was not suitable, and 30 min was selected.

To sum up, the optimal conditions for SW extraction were 50 mL of sample adjusted to pH 2 with 100 mg of Q-100 MPs stirred at 900 rpm for 30 min.

3.1.2. Elution conditions

Due to the low magnetization of the particles, the elution was carried out in the filtration assembly, which was used to separate the particles from aqueous sample,

252 while the retained analytes were eluted by passing a volume of the elution solvent
253 through the same assembly.

254 The elution strength of MeOH and ACN was compared and, under the same
255 conditions, ACN provided lower extraction recoveries compared to MeOH, due to the
256 different selectivity. Therefore, MeOH was maintained.

257 As regards the volume, three fractions of 5 mL MeOH each were passed through
258 the Q-100 MPs. With the first 5 mL, recoveries ranged from 56% to 78% for all the
259 analytes, except ACE (12%), ASP (44%) and ALI (43%). In the second fraction of
260 MeOH, the recoveries were between 5% and 10% for all the compounds. Lastly, with
261 the third fraction of MeOH, no improvement in recoveries was observed. In this
262 respect, previous studies dealing with the extraction of SWs by SPE [6, 25] also
263 pointed out that the use of pure MeOH and ACN was not sufficient to elute out certain
264 SWs, such as ACE, SAC, NEO and STV. The improvement in the recoveries of these
265 compounds had been achieved with basic additives (i.e. NH_4OH) in the solvent [6, 25].
266 Thus, when 5 mL of MeOH followed by 5 mL of 2% NH_4OH in MeOH were used, the
267 extraction recoveries increased from 16% to 40% for the abovementioned analytes,
268 while the rest of analytes did not show any improvement. Thus, all the studied SWs
269 can be desorbed with recoveries up to 75%, except ACE (32%), ASP (60%) and ALI
270 (50%). Considering the obtained results, 5 mL of MeOH followed by 5 mL of 2%
271 NH_4OH in MeOH (10 mL volume) was chosen.

272 The 10 mL of elution solvent was evaporated to dryness using a miVac
273 concentrator and the residue was re-dissolved in 1 mL of ultrapure water and MeOH
274 (9:1, v/v) before injecting to LC. During this step, less than 5% losses were observed
275 for all compounds.

After the optimization of the d-SPE procedure, the conditions used for further application in real samples were as described in Section 2.3. Under the optimal conditions, the extract from d-SPE was injected into the LC-MS/MS, in which CYC and SUC could be monitored and provided recovery values of 36% and 98%, respectively. Thus, on the whole, the recovery values (%) for each analyte in ultrapure water were in the range of 60% to 98% for all compounds, expect for ACE, CYC and ALI, which were 32%, 36% and 50%, respectively. A possible explanation could be that the MPs coated with non-polar polystyrene-based material was not able to retain the more polar analytes of this challenging group. It should be noted that the sorption capacity of Q-100 MPs is rather affected from the initial capacity [23], and these particles can be reused up to 30 extractions.

In fact, the present results are comparable to those obtained in a previously studies [6, 25, 26], where higher amount of the commercial available Oasis HLB (500 mg) [25] and HR-X (500 mg) [26] sorbent were evaluated for the extraction of SWs in 100 mL [25] and 500 mL [26] of water sample. It is clear from the results that, with a small amount of Q-100 material, better recoveries might be achieved because of the hypercrosslinked structure of Q-100 that provides high surface area to interact with the extracted analytes.

3.2 Method validation

The Q-100 material was applied for the extraction of the SWs from environmental water samples, including river water, and effluent and influent samples from a WWTP.

Table 1 shows the apparent recovery (%R_{app}) and matrix effects (%ME) that were calculated for each kind of sample. Sample volumes of 50 mL for river water and 25 mL for effluent and influent wastewaters were selected due to the complexity of the

matrix and to reduce the %ME. The $\%R_{app}$ and %ME were calculated at low and high concentration levels for all matrices except influent wastewater, which was just calculated at the high concentration level. Firstly, non-spiked samples were analyzed in order to subtract the signal of existing analytes.

The $\%R_{app}$ were calculated by comparing the responses of pre-spiked sample with the responses of pure standard solution at same concentration. As can be seen in Table 1, in river water samples at both levels of concentration, it was observed that $\%R_{app}$ were higher than 70% for all the analytes, except for ACE, CYC, ASP, which were between 21% and 33%, and ALI, which was below 12%. In effluent wastewater at both levels, it was also observed that $\%R_{app}$ for ACE, CYC, ASP and NHDC were below 24% and, for rest of analytes, ranged from 30% to 69%. As expected, lower values of $\%R_{app}$ were obtained in influent wastewater than in river and effluent wastewater due to the complexity of matrix. The obtained $\%R_{app}$ for all studied analytes ranged from 18% to 45%, except for CYC, ASP and NHDC, which were below 10%.

The ME was evaluated in the three types of matrices and was calculated by comparing the signal response obtained when spiking a sample after extraction with the signal response obtained from a standard solution at the same concentration. If the %ME=0, no ME is present, if %ME<0, there is ion suppression and if %ME>0, there is ion enhancement. Ion suppression was observed for all analytes in all matrices (data shown in Table 1). In river water, similar ion suppression was observed at the two levels of concentration, which was less than 36% for all analytes. However, in effluent wastewater samples ion suppression was higher with values from 37% to 65% for all analytes, except for ACE and SAC, which had values lower than 26% and 14%,

respectively. In the case of influent wastewater, high ion suppression was observed for ASP, ALI, NHDC, STV, NEO and GLY (values ranged from 63% to 88%) but, for the other analytes, the values of ion suppression ranged from 23% to 39%, which is fairly good and similar to river and effluent matrices. These results might be attributed to the high content of compounds in the sample that are strongly retained by Q-100 MPs which is a high retentive material with lack of selectivity, and eventually affect the analytes' ionization.

Then, the analytical method based on d-SPE/LC–MS/MS was validated including the following parameters: linearity, repeatability, reproducibility, limits of detection (LODs), and limits of quantification (LOQs). For river water, matrix-matched calibration curves were plotted and the linear range (details in Table 2) of was very suitable, whereas for effluent samples the instrumental calibration curve and applying the corresponding $\%R_{app}$ was assessed, and they ranged from 0.02 to 2 µg L^{-1} with determination coefficients (R^2) in both cases greater than 0.9995. The LODs for the compounds present in the samples (ACE, SAC, CYC and SUC) were calculated on the basis of the ILODs and applying $\%R_{app}$, and they ranged between 0.004 and 0.02 µg L^{-1} for river samples, and 0.01 and 0.05 µg L^{-1} for effluent. LODs for rest of the compounds were calculated as S/N 3:1, and they ranged between 0.001 and 0.005 µg L^{-1} for river samples, and 0.002 and 0.01 µg L^{-1} for effluent samples. The LOQs were selected as the lowest point of calibration curve. The repeatability and reproducibility between days were both measured at 0.05 µg L^{-1} and 0.5 µg L^{-1} concentration levels (and details for river water are in Table 2, and similar values were obtained when effluent water samples were analyzed.

3.3 Application to environmental samples

To evaluate the applicability of the d-SPE/LC–MS/MS method, three different samples of river and effluent wastewater were analyzed in triplicate and the quantitative results are shown in Table 2S. The identification of compounds in the sample was based on the retention time and ratios between the quantifier and qualifier transitions, when compared with those of standards. ACE, SAC, CYC, and SUC were found in all river and effluent water samples analyzed. However, the rest of SWs were not detected in any of the samples analyzed, which is in line with previous studies [6, 25].

As regards river water, trace levels of ACE, SAC and SUC were found (from 0.1 to 0.4 μg L^{-1}), whereas CYC was detected at a concentration below its LOQ. As for effluent wastewater, the same analytes were present, but at higher concentrations (from 4.7 to 17.9 μg L^{-1}) except in the case of SAC, which was at similar concentrationthan that in river (0.1 - 0.2 μg L^{-1}), and CYC, which was also found at a concentration below its LOQs. The fact that these analytes were present in river water could be explained for their incomplete elimination at WWTPs. The concentrations detected in this study were similar to previous studies [6, 25] in which water supplied from the same WWTPs was analyzed, although higher concentration levels were found in wastewater samples in North-West Spain [12] and Switzerland [27].

4. Conclusions

The evaluated new material Q-100 MPs with hypercrosslinked properties provided high retention features with respect to SWs. However, the limited magnetization should be addressed to be able to exploit Q-100 MPs fully.

The magnetization of the particles should be improved through changing the modifier, reducing the particle size of Q-100 or increasing the proportion of the Fe_3O_4 nanoparticles.

Under optimized conditions, the recoveries of analytes in different environmental samples were comparable with previous results obtained with SPE using commercially available sorbents, although they were highly affected by the matrix effect.

The validated method based on d-SPE/LC-MS/MS was applied to the determination of SWs in river water samples and effluent wastewater samples, where ACE and SUC were the analytes found at higher concentrations.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Figure Captions

Figure 1. Effect of the sample pH on extraction recovery of the analytes using Q 100 MPs in d-SPE (%RSD (n=3) were lower than18%).

Table 1. %R_{app} and %ME of SWs in river and effluent wastewater samples by d-SPE extraction techniques.

Analyte	River water (50 mL)				Effluent (25 mL)				Influent (25 mL)	
	spiked at 0.05 (µg L^{-1})		spiked at 0.5 (µg L^{-1})		spiked at 0.1 (µg L^{-1})		spiked at 1 (µg L^{-1})		spiked at 0.4 (µg L^{-1})	
	% R_{app}	% ME	% R_{app}	% ME	% R_{app}	% ME	% R_{app}	% ME	% R_{app}	% ME
ACE	31	-3	27	-21	20[a]	-26	24[b]	-21	18[b]	-23
SAC	70	-6	75	-4	69	-9	63	-14	34	-39
CYC	21	-29	27	-33	5	-49	16	-46	4	-27
ASP	30	-35	33	-36	19	-52	18	-74	2	-57
SUC	84	-21	78	-24	43[a]	-37	50[b]	-31	45[b]	30
ALI	12	-13	11	-15	38	-39	42	-32	25	-74
NHDC	70	-27	72	-20	24	-61	23	-70	9	-88
STV	88	-16	84	-12	43	-56	30	-65	22	-78
NEO	72	-27	75	-28	35	-64	30	-68	20	-78
GLY	85	-29	69	-22	66	-20	57	-34	23	-63

[a] Spiked at 1 µg L^{-1} (n=3)

[b] Spiked at 2 µg L^{-1} (n=3)

Table 2. LODs, linear range, repeatability and reproducibility between days obtained when 50 mL of river water sample spiked at 0.05 µg L^{-1} and 0.5 µg L^{-1} of each analyte were analyzed by d-SPE-LC–MS/MS.

Analyte	LODs (µg L^{-1})	Linear range (µg L^{-1})	Repeatability (%RSD, n=5)		Reproducibility (%RSD, n=5)	
			0.05 (µg L^{-1})	0.5 (µg L^{-1})	0.05 (µg L^{-1})	0.5 (µg L^{-1})
ACE	0.006[a]	0.05 - 1	16	6	13	15
SAC	0.01[a]	0.05 - 1	7	3	16	12
CYC	0.004[a]	0.02 - 1	15	2	18	10
ASP	0.002	0.02 - 1	14	6	17	15
SUC	0.02[a]	0.05 - 1	12	7	14	14
ALI	0.005	0.01 - 1	8	19	19	14
NHDC	0.001	0.01 - 1	5	8	7	7
STV	0.001	0.01 - 1	6	3	3	7
NEO	0.001	0.01 - 1	5	3	4	7
GLY	0.003	0.02 - 1	9	6	10	4

[a] Calculated from instrumental LODs considering apparent recovery

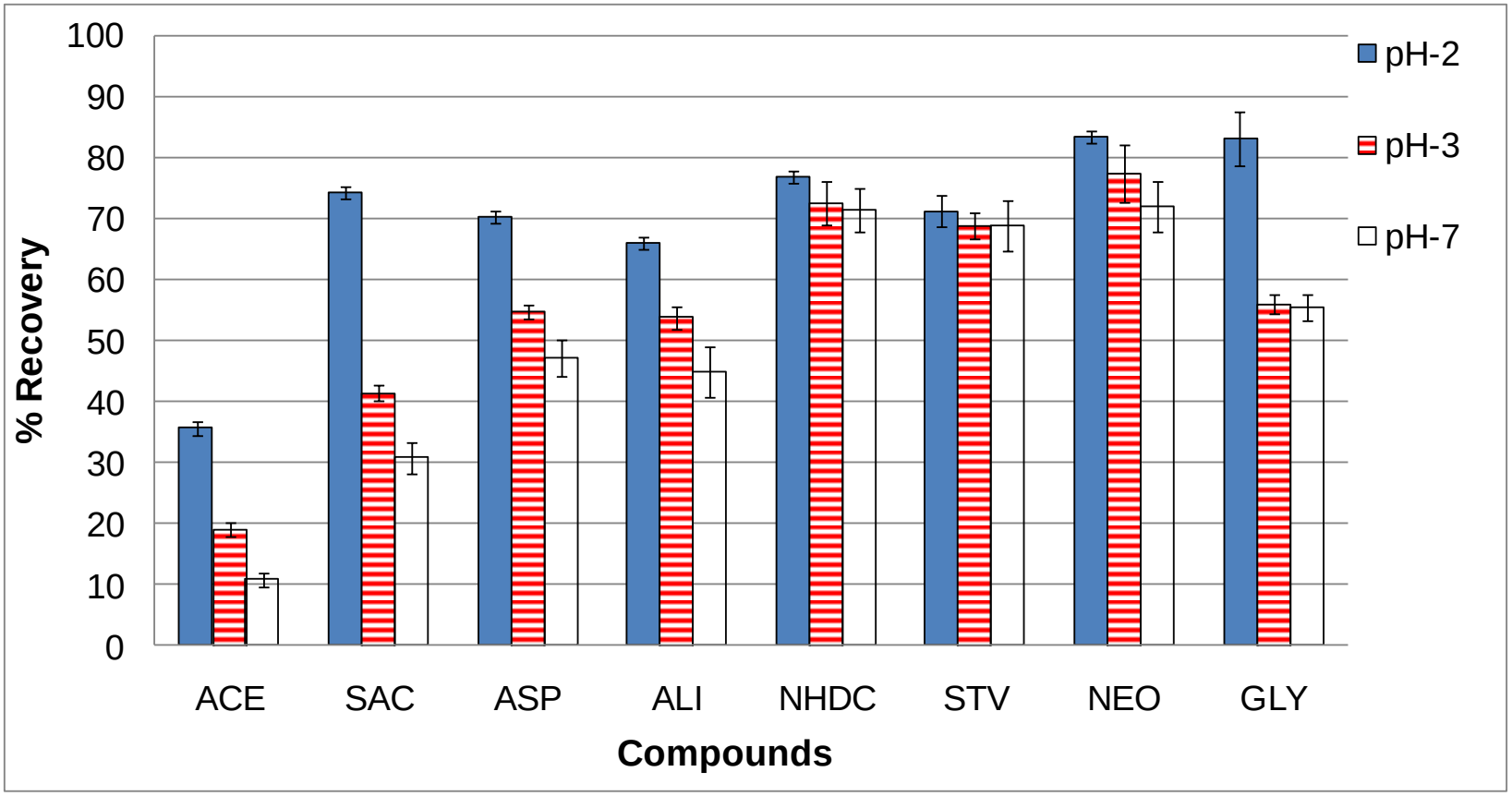
100
90
80
70
60
50
40
30
20
10
0
% Recovery
pH-2
pH-3
pH-7
ACE
SAC
ASP
ALI
NHDC
STV
NEO
GLY
Compounds

Figure 1