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Oxidized multi-walled carbon nanotubes for the dispersive solid-phase extraction of quinolone antibiotics from water samples using capillary electrophoresis and large volume sample stacking with polarity switching

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ABSTRACT

In this work, a new method for the determination of eleven quinolone antibiotics (moxifloxacin, lomefloxacin, danofloxacin, ciprofloxacin, levofloxacin, marbofloxacin, enrofloxacin, difloxacin, pefloxacin, oxolinic acid and flumequine) in different water samples using dispersive solid-phase extraction (dSPE) and capillary zone electrophoresis with diode-array detection was developed. Oxidized multi-walled carbon nanotubes (o-MWCNTs) were used for the first time as stationary phases for the off-line preconcentration by dSPE of the antibiotics. A 65 mM phosphate buffer at pH 8.5 was found adequate for analyte separation while large volume sample stacking with polarity switching of the analytes dissolved in water containing 10% (v/v) of acetonitrile was carried out in order to improve the sensitivity. dSPE parameters, such as sample volume and pH, o-MWCNT amount, volume and type of eluent in dSPE were optimized. Application of the developed method to the analysis of spiked Milli-Q, mineral, tap, and wastewater samples resulted in good recoveries values ranging from 62.3 to 116% with relative standard deviation values lower than 7.7% in all cases. Limits of detection were in the range of 28–94 ng/L. The proposed method is very fast, simple, repeatable, accurate and highly selective.

1. Introduction

In the last decade, special concern has arisen regarding the occurrence and fate of emerging contaminants like surfactants, personal care products, industrial additives, pharmaceuticals, and a good number of chemicals purported to be endocrine disrupters in the environment [1]. Their monitoring in water resources should be an important health and safety matter, especially when the existing conventional water treatment plants were not initially designed for their elimination and no specific regulation already exists in this sense.

Quinolones constitute an important class of pharmaceutical compounds with a broad spectrum activity that act by the inhibition of the ADN-gyrase. Their consumption for both human and veterinary purposes is currently increasing and as a result, they have also been found in environmental waters, especially ofloxacin (OFLO), lomefloxacin (LOME), norfloxacin (NORFLO) and ciprofloxacin (CIPRO) [2–8]. Their analysis is frequently developed by HPLC [9–16] although CE applications have also been proposed [2,4,17–19]; however, the literature dealing with the analysis of quinolones in environmental waters is still sparse. In this sense, the number of studies using CE is even more limited [2] probably because of the low sensitivity of the technique, which is associated to the injection of low volumes of sample and to the short optical path-length if optical detection is used. To overcome this limitation, on-line preconcentration strategies, also known as stacking or sweeping techniques [20–22] have been developed, which take advantage of differences in mobility and conductivity between sample and buffer. They can be easily employed since no special devices are required. However, up to now very few works have applied them with success for the on-line preconcentration of quinolone antibiotics [23–26]. He et al. [23], for example, used field-amplified sample stacking for the CE-UV analysis of nine fluoroquinolones in chicken, while Altria and Chanter [24] used pH mediated sample stacking for the preconcentration of a single quinolone antibiotic. Hernández et al. [25] also compared the use of field-enhanced sample injection with isotachophoresis for the on-line preconcentration of one quinolone (marbofloxacin,

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MARBO) from pig plasma extract, while in a second work of the same group, Hernández et al. [26] also used isotachophoresis for the determination of three quinolones (enrofloxacin, ENRO, CIPRO and flumequine, FLUME) in the same samples. Therefore and despite their inherent benefits, there is an important gap in the literature concerning the use of stacking techniques in CE to improve the selectivity and sensitivity for this group of compounds as well as to increase the number of studied quinolones.

Two of the stacking techniques most commonly applied in CE and which have not been used for the preconcentration of quinolones are normal stacking mode (NSM) and large volume sample stacking (LVSS). The first of them [20] constitutes the simplest of the stacking modes. In this case, the sample which is dissolved in a low conductivity matrix is hydrodynamically injected into the capillary. Focusing happens at the interface between the BGE and the sample matrix due to a drastic change in the electrophoretic velocity. Concerning LVSS [21,27], in which only cations or anions can be concentrated at one time, the sample volume injected is much bigger than in NSM. In this case, the sample matrix is pumped out of the capillary moving towards the inlet maintaining the stacked analytes on the sample/BGE interface by manipulation of the EOF which can be done with or without polarity switching. In the first situation, polarity is switched and the current monitored until it reaches 90–99% of the value obtained when the capillary is only filled with the BGE. At this point, the polarity is switched so that the EOF direction is towards the detector. In the second case, for anions it is enough to suppress the EOF with a suitable BGE additive while for cations it is also necessary to reversed the EOF with an appropriate EOF modifier.

Carbon nanotubes (CNTs) are relatively new nano-materials that have found an extensive variety of applications in analytical sciences. In particular, the role of CNTs in analytical chemistry is becoming more important due their demonstrated applications [28], which also involve their use as stationary phases in solid-phase extraction (SPE). In this sense, CNTs have revealed to have an adequate sorption capacity for the extraction of both organic and inorganic compounds as shown in a recent review article [29]. From a detail revision of the literature, it is also clear that few works have used CNTs-SPE in combination with CE analysis [30] and regarding the specific use of CNTs for the SPE of pharmaceuticals from water samples, the number of applications developed till now is also relatively low. Up to now, they have only been used for the extraction of tetracyclines from surface waters [31] (using multiwalled CNTs, MWCNTs, of 20–50 nm diameter and 5–20 μm long), sulfamides and cephalosporins from tap and well water [32] (using MWCNTs of 30–60 nm diameter), sulfonamides from eggs and pork after extraction with ACN, ferrite potassium cyanide and zinc sulfate [33] (using MWCNTs of 5–15 μm long and diameter of 60–100 nm) and chloramphenicol from eggs, honey and milk after a suitable dilution with water at 60 °C [34] (using MWCNTs of 5–15 μm long and diameter of 40–60 nm). While concerning quinolone extraction, up to now, only one work has used then for this purpose [35]. However, in this case magnetic nanoparticles (MNPs) were deposited onto MWCNT surface to simplify sample treatment, which were later used for the extraction of ten of these analytes (i.e. enoxacin, NORFLO, OFLO, CIPRO, danofloxacin, DANO, LOME, ENRO, difloxacin, DIFLO, cinoxacin and nalidixic acid) from human plasma. The use of free MWCNTs (not modified with MNPs) of 110–170 nm diameter and 5–9 μm length, revealed that such CNTs (which are one of the longest commercialized nowadays) could be used for the extraction of these analytes; however, a full study regarding the applicability of these or other nanotubes for real sample analysis especially waters, has not yet been developed.

Therefore, the aim of this work is to study the application of MWCNTs of 110–170 nm diameter and 5–9 μm length, specially their oxidized form, for the extraction of eleven quinolone antibiotics (moxifloxacin, MOXI, LOME, DANO, CIPRO, levofloxacin, LEVO, MARBO, ENRO, DIFLO, pefloxacin, PEFLO, OXO and FLUME) from different types of water samples. CE with DAD has been used in combination with LVSS with polarity switching as on-line preconcentration technique in order to improve instrumental sensitivity. For the extraction and preconcentration of the selected quinolones from water samples, a SPE procedure was developed in the dispersive mode. Although, dSPE is normally used for matrix clean-up purposes, which means that the dispersive sorbent is added to the bulk solution or matrix containing the analytes and the possible matrix interferences/components are retained onto it to finally discard the sorbent and analyze the supernatant, it can also be used with the aim of trapping the target analytes which are later eluted or desorbed with an appropriate solvent [36–38]. In this last case which is not so widely used, the extraction time is considerably reduced and at the same time, the whole procedure is simplified becoming faster and easier than conventional SPE. Concerning the particular use of CNTs for SPE purposes, up to now and, to the best of our knowledge, only one work dealing with the extraction of pesticides and which has also been developed by our group [39], has used CNTs in the dSPE mode, providing good results. Besides, this is the first work in which oxidized-MWCNTs (o-MWCNTs) are used as stationary phases for the extraction and preconcentration of quinolone antibiotics from water samples and also the first work dealing the use of LVSS with polarity switching for the on-line preconcentration of quinolones in CE.

2. Experimental

2.1. Chemicals and samples

All chemicals were of analytical reagent grade and used as received. HPLC grade methanol, acetonitrile (ACN) and acetone were purchased from Panreac Química S.A. (Barcelona, Spain), while HPLC grade dichloromethane was provided from Scharlau (Barcelona, Spain). Sodium dihydrogen phosphate dihydrate, hydrochloric acid, nitric acid, ammonium hydroxide, formic acid and diethyl ether were obtained from Merck (Darmstad, Germany). Sodium hydroxide was from Fluka (Madrid, Spain). Distilled water was deionized by using a Milli-Q gradient system A10 (Millipore, Bedford, MA, USA). MWCNTs with an average diameter of 110–170 nm and 5–9 μm length were provided by Sigma–Aldrich (Madrid, Spain). Empty glass SPE tubes of 6 mL volume and PTFE frits (20 μm porosity) were from Supelco (Madrid, Spain).

Analytical standards of LOME hydrochloride, DANO, CIPRO, LEVO, MARBO, ENRO, DIFLO hydrochloride, PEFLO, OXO and FLUME were purchased from Sigma–Aldrich. MOXI was kindly supplied by Bayer Healthcare (Barcelona, Spain). Standards were used without further purification (purity >97%). Mixture stock solution of the quinolones of approximately 100 mg/L were prepared by dissolving them in methanol and stored in the darkness at 4 °C. Working standard solutions for the calibration curves and the fortifications assays were prepared daily by suitable dilution.

Milli-Q water (pH 5.0, conductivity of 4.06 μS/cm at 25 °C) was taken from a Millipore system. Mineral water (pH 8.5, conductivity of 320 μS/cm at 25 °C) was purchased from a local supermarket of Tenerife (Canary Islands, Spain). Tap water (pH 7.6, conductivity of 483 μS/cm at 25 °C) was taken from the city of Tacoronte (Tenerife, Spain), while wastewaters (pH 8.1 and 7.8, conductivities of 2.44 and 2.63 mS/cm at 25 °C, biological oxygen demand (BOD) = 10 and 10.5 mg oxygen/L) were collected from a wastewater treatment plant from Las Americas (Tenerife, Spain). Before use, wastewaters were filtrated through Durapore membrane filters (0.45 μm) of polyvinylidene fluoride (PVDF) from Millipore in order to remove any solid particle. Water samples (Milli-Q, mineral,

tap and wastewaters) were spiked with the selected antibiotics at several concentrations.

2.2. CE-DAD

CE-DAD analyses were performed in a P/ACE 5510 CE system (Beckman Instruments, Fullerton, CA, USA), equipped with a DAD working at 250 nm (for OXO and FLUME) and 280 nm (for the rest of the quinolones). System Gold Software was used for instrument control. Fused silica capillaries (Composite Metal Services, Shipley, West Yorkshire, UK) with 75 μm internal diameter were used, being the detection length to the DAD detector 60 cm and the total length 67 cm. Before its first use, each capillary was washed using nitrogen pressure at 20 psi (1 psi = 6894.76 Pa) for 2 min with 1.0 M HCl, 2 min with water, 5 min with 0.1 M NaOH, 2 min with water and 2.5 min with optimum running buffer (65 mM phosphate buffer at pH 8.5). Capillary conditioning was performed daily by rising at 20 psi with water for 1 min and with BGE for 2 min. To achieve a good repeatability between runs, 1 min washing with water and 2 min more with BGE (all using 20 psi) was carried out. At the end of the day, the capillary was rinsed with water for 3 min. Electrophoretic separation was carried out at 25 °C and +15 kV. Injection volumes were determined using the CE expert software from Beckman Coulter.

2.3. Conditions for LVSS with polarity switching

LVSS with polarity switching conditions were the following: the capillary was first filled with the BGE, then a large plug of sample (analytes were dissolved in water with 10%, v/v ACN) was hydrodynamically injected for 3 s at 20 psi. A high voltage (−15 kV) was then applied and the electric current was monitored to control sample matrix removal from the capillary. When the current become 95–99% of the value obtained with the BGE the voltage was turned off and the polarity was reversed to run the separation.

2.4. Oxidation of MWCNTs

The o-MWCNTs were prepared by a slight modification of a previously published procedure [40]. Briefly, 10 g of pristine MWCNTs was suspended in 1.0 L of 3.0 M HNO_3, sonicated for 30 min using a Raypa® Model UCI-150 ultrasonic cleaner from R. Espinar S.L. (Barcelona, Spain) and stirred at 80 °C and 500 rpm for 24 h using a magnetic stirrer Agimatic-E from J.P. Selecta (Barcelona, Spain). Then, the black suspension was diluted with Milli-Q water to 2.0 L, filtered through a 0.22 μm and 47 mm nylon membrane (Sigma–Aldrich, Madrid, Spain) and washed with Milli-Q water until neutral pH. Afterwards, the black solid was washed twice with diethyl ether and dried overnight under vacuum at room temperature. The final oxidation yield was 94.7%.

2.5. Conductivity and BOD measurements

The conductivity was directly measured into the filtrated samples with a Crison CM 35 portable conductimeter (Crison Instruments SA, Barcelona, Spain) with temperature measurement capability. BOD was calculated from the decrease in dissolved oxygen concentration over a 5 day period (BOD_5) following the UNE-EN-1899 norm.

2.6. dSPE procedure

250 mL of spiked sample (Milli-Q, mineral, tap and wastewaters) was adjusted to pH 5.0 with 0.1 M HCl and transferred to a flask containing 150 mg of o-MWCNTs. The flask was tightly capped and shaken for 10 min. After the extraction, the dispersed o-MWCNT solution was passed through a glass SPE tube containing inside a PTFE frit, using a Visiprep™-DL SPE vacuum manifold from Supelco (Bellefonte, PA, USA). Then, a new frit is introduced into the glass tube in order to hold the stationary phase with the retained analytes. For the wastewater samples, washing with 2.0 mL of Milli-Q water was carried out after the loading of the sample into the glass tube. Afterwards, vacuum of −10 mmHg for 15 min was applied in order to dry the cartridge. The retained quinolones were eluted with 25 mL of 3:1 (v/v) acetone/methanol, coupling a 0.20 μm filters outside the glass tube (Chromafil® Xtra PET-20/25), and the organic solvent was then evaporated to dryness at 40 °C and 270 mbar using a rotavapor R-200 (from Büchi Labortechnik, Flawil, Switzerland) equipped with a vacuum controller V-800 and a vacuum pump V-500 (also from Büchi Labortechnik). The dry residue was redissolved in 1.0 mL of Milli-Q water containing 10% (v/v) ACN (stacking solvent), filtrated using 0.20 μm filters (Chromafil® Xtra PET-20/25 from Macherey-Nagel) and then analyzed by CE-DAD following optimum LVSS conditions.

3. Results and discussion

3.1. CE-DAD method

Among the different quinolones selected for this study nine of them (MOXI, LOME, DANO, CIPRO, LEVO, MARBO, ENRO, DIFLO and PEFLO) belong to the fluoroquinolone family, which have a fluor atom in 6th position and a piperazinyl moiety in 7th position. Since most fluoroquionolones have two relevant ionizable functional groups, their acid-base chemistry involves two equilibria: the dissociation of the carboxylic group (pK_{a1} in the range 5.0–6.5) and the deprotonation of the N4 of the piperazine ring placed at position 7 (pK_{a2} in the range 6.0–8.5) [41–45]. At pH values between their pK_{a1} and pK_{a2} they are in their zwitterionic form. In the case of OXO and FLUME, which are not fluoroquinolones, they only have one ionizable group (dissociation of the carboxylic acid) with pK_a values of 6.78 [44] and 6.61 [46], respectively. Thus, according to their ionic nature, they show better separation by CZE at basic pH than acidic or stronger alkalinic pH [47–50]. As a consequence, in the current work, the optimization of the separation was carried out using the common basic buffer system, sodium dihydrogen phosphate-sodium hydrogen phosphate covering the pH range of 7.0–9.0 and a concentration range between 50 and 100 mM. All the experiments were carried out hydrodynamically injecting for 5.0 s at 0.5 psi a mixture of the analytes dissolved in methanol containing 5.0 mg/L of each antibiotic (higher injection times could not be achieved). Fig. 1 shows the electropherograms obtained when different concentrations of phosphate were used at pH 8.5. As can clearly be seen, all analytes were effectively separated in less than 19 min using a 65 mM phosphate buffer at pH 8.5 with a separation voltage of +15 kV, which provided a good compromise among peak shape, resolution and electrical current intensity. Previous works in the literature have also used this type of buffer system alone [49,51] or with some organic modifiers [17,52] with good results. Under these conditions, limits of detection (LODs) achieved (calculated on the basis of a signal that is three times baseline noise measurement) were in the range 114–225 μg/L.

3.2. Stacking procedures

With the aim of enhancing the sensitivity obtained with the CE-DAD method, two common stacking strategies were investigated: NSM, which is the simplest among the stacking modes, and LVSS with polarity switching. Since stacking techniques normally provide an important sensitivity improvement, initial experiments were carried out using mixtures of the selected quinolones dis-

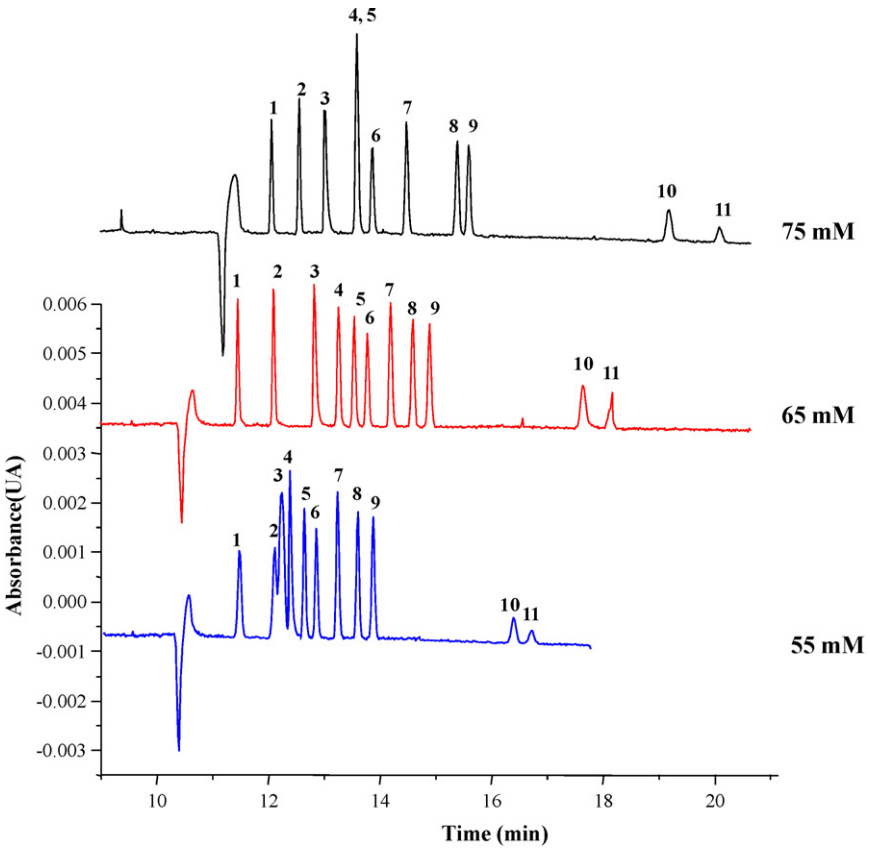

Fig. 1. Influence of the concentration of phosphate in the BGE (pH 8.5). Injection: 5.0 s at 0.5 psi. Sample in methanol (5 mg/L of each analyte). Total length of the capillary: 67 cm (60 cm effective length). Voltage: +15 kV. Temperature: 25 °C. Detection at 280 nm. Peak identification: (1) MOXI, (2) LOME, (3) DANO, (4) CIPRO, (5) LEVO, (6) MARBO, (7) ENRO, (8) DIFLO, (9) PEFLO, (10) OXO and (11) FLUME.

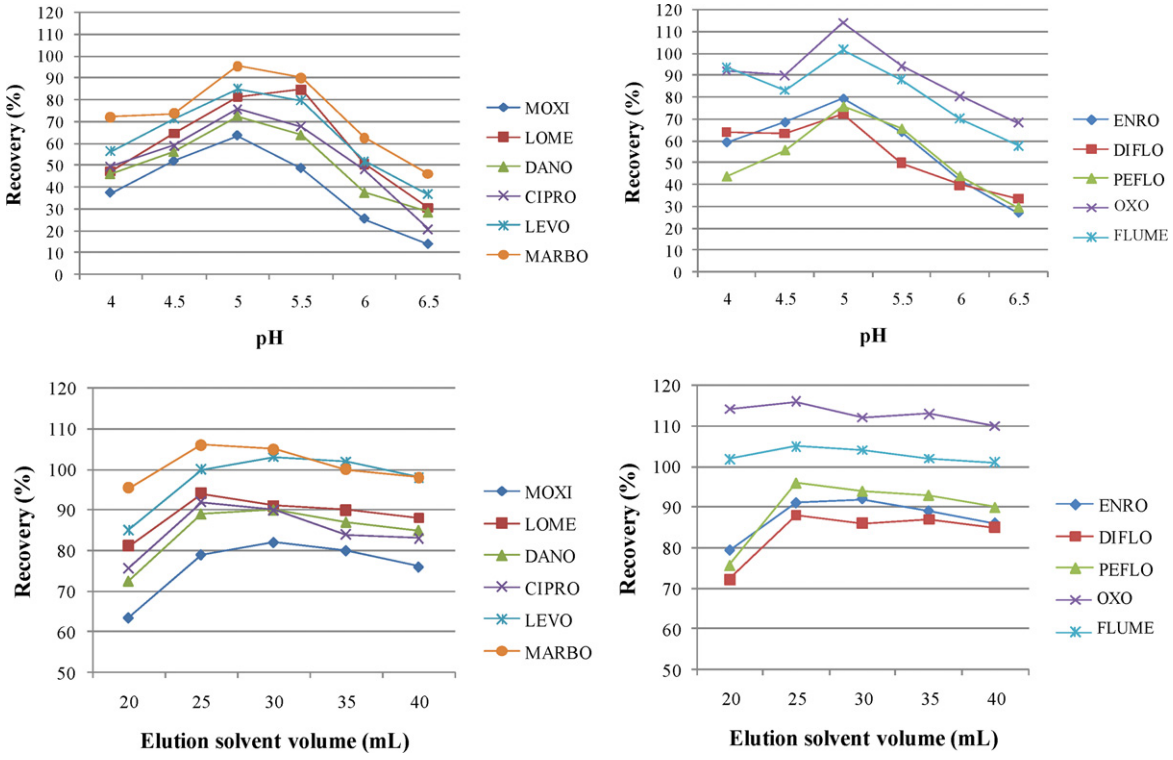

Fig. 2. Top: Effect of the sample pH on the extraction of the eleven antibiotics from water samples ($n = 2$ in each case). Extraction conditions: 25 mg of o-MWCNTs, 25 mL of Milli-Q water (10 μg/L of each analyte), 20 mL of 3:1 (v/v) acetone/methanol as elution solvent. Down: Effect of the volume of the elution solvent (3:1 (v/v) acetone/methanol) on the recoveries of the eleven antibiotics ($n = 2$ in each case). Extraction conditions: 150 mg of o-MWCNTs, 250 mL of Milli-Q water (1.0 μg/L of each analyte) at pH 5.0.

Table 1
Results of the repeatability study (expressed as RSD percentage) obtained for the LVSS-CE-DAD procedure (data given for 250 μg/L) and calibration data for the selected quinolones.

Peak	Antibiotic	Intraday precision ($n=3$)		Interday precision ($n=15$)		Calibration data ($n=8$)				LOD (μg/L)[a]
		t_m	Area	t_m	Area	Range of concentration tested (mg/L)	b (S_b)	a (S_a)	R^2	
1	MOXI	0.1	3.5	0.3	5.6	0.078–0.441	0.381 (0.005)	−0.008 (0.001)	0.999	14.6
2	LOME	0.1	3.4	0.4	2.5	0.089–0.432	0.572 (0.013)	−0.014 (0.004)	0.997	16.7
3	DANO	0.1	0.8	0.5	4.0	0.038–0.360	0.863 (0.017)	−0.007 (0.004)	0.998	7.19
4	CIPRO	0.1	6.4	0.5	2.3	0.051–0.377	0.632 (0.015)	0.004 (0.004)	0.997	9.55
5	LEVO	0.1	0.3	0.6	4.2	0.060–0.432	0.545 (0.012)	−0.004 (0.003)	0.997	11.2
6	MARBO	0.1	2.5	0.6	4.4	0.064–0.427	0.498 (0.007)	−0.009 (0.002)	0.999	12.1
7	ENRO	0.1	5.6	0.5	2.9	0.072–0.448	0.753 (0.015)	−0.012 (0.004)	0.997	13.5
8	DIFLO	0.1	2.9	0.7	3.6	0.076–0.422	0.706 (0.009)	−0.016 (0.003)	0.999	14.2
9	PEFLO	0.1	2.0	0.6	2.2	0.085–0.450	0.631 (0.016)	−0.016 (0.005)	0.996	16.0
10	OXO	0.2	2.9	0.5	5.3	0.062–0.454	0.816 (0.018)	0.009 (0.005)	0.997	11.6
11	FLUME	0.1	2.2	0.6	2.7	0.040–0.447	1.319 (0.030)	0.004 (0.009)	0.997	7.42

b, slope; S_b, SD of the slope; a, intercept; S_a, SD of the intercept; R^2, determination coefficient.
[a] Calculated as three times the S/N.

solved in different solvents at a concentration of approximately 250 or 500 μg/L.

Concerning NSM [20] a large sample plug of a low conductivity is firstly introduced in the CE system. Considering anionic analyte separation under normal polarity (like in this case), when voltage is applied to both ends of the capillary, the existence of a higher electric field in the low conductivity sample makes anions move towards the anode until they reach the boundary between the sample solution and the BGE In the current work, different mixtures of water and BGE were tested, as well as solutions containing water with 5–70% (v/v) of ACN (values higher than 70% generated current breakdowns). Such mixtures, as well as ACN have also shown to provide good results in this sense [53–55], in particular, the use of ACN as additive has demonstrated a significant increase in the stacking of basic [56] and cationic compounds [57]. In addition, quinolones were also dissolved in either water or BGE alone. Analyte mixtures were injected between 10 and 35 s at 0.5 psi. Among these solutions, the use of water alone provided the best results. With the increase of the injection time, peak heights also increased (as expected) but at the same time deterioration of the separation efficiency was observed, due to the high amount of solvent injected. Thus, the sample could only be introduced in the capillary up to 20 s at 0.5 psi, which is approximately a 3% of the capillary volume. Under these conditions, LODs obtained were in the range 43–126 μg/L, which corresponds to a sensitivity improvement between 2 and 3 times compared with a non-stacking injection.

In order to further improve these results, a second stacking procedure was investigated, namely, LVSS [20]. A limitation of LVSS is that only positive or negative solutes can be effectively concentrated at a time. Under normal polarity separation conditions, only anionic species like the ones of this work can be separated since a cathodal EOF is necessary. In this case, sample enrichment is also obtained by the hydrodynamic injection of a sample with a lower conductivity than that of the BGE. Following the hydrodynamic sample injection, the sample vial is replaced with the BGE vial and reversed/negative voltage is then applied (−15 kV in this case). A lower electrical current is initially observed that increases progressively while the cationic and neutral species of the sample are pumped out of the capillary. When 95–97% of the original current is reached (also called reversal time), the negative voltage is stopped and the polarity is switched to develop the separation. At this specific point, anionic analytes still remain in the capillary and are later separated once positive voltage is again applied. For the application of the LVSS procedure both the conductivity of the matrix and the injection time should be optimized. For this purpose, 250 μg/L the quinolones dissolved in different mixtures of water and BGE; water with 5–70% (v/v) of ACN and water or BGE alone were also tested. The use of water with 10% (v/v) of ACN provided the highest preconcentration of the analytes, allowing the injection of the sample up to 3.0 s for 20 psi (which is approximately 18% of the capillary volume) without loss of resolution and peak distortion. Reversal time was found to be 1.1 min. The application of higher injection times provided an important decrease in resolution. Under optimum separation and stacking conditions the LODs achieved (calculated as three times the signal-to-noise (S/N) ratio) ranged between 7.19 and 16.7 μg/L which are up to 20 times lower compared with non-stacking conditions. Such sensitivity improve-

Table 2
Mean recovery percentages ($n=3$) of the repeatability study obtained for the MWCNT oxidation. Spiked Milli-Q water at 1.0 μg/L was used in all assays.

Peak	Antibiotic	Oxidation 1 Mean recovery ($n=3$) and RSD (%)	Oxidation 2 Mean recovery ($n=3$) and RSD (%)	Oxidation 3 Mean recovery ($n=3$) and RSD (%)	Oxidation 4 Mean recovery ($n=3$) and RSD (%)	Oxidation 5 Mean recovery ($n=3$) and RSD (%)	Mean recovery ($n=15$)	RSD ($n=15$, %)
1	MOXI	81.4 (4.2)	73.8 (2.9)	73.8 (2.9)	75.3 (1.0)	71.0 (5.1)	74.9	5.8
2	LOME	94.7 (1.9)	90.6 (7.0)	90.6 (7.0)	86.6 (3.8)	89.5 (9.4)	89.7	5.7
3	DANO	90.4 (1.8)	85.4 (9.8)	85.4 (9.8)	84.5 (6.4)	84.9 (3.2)	86.0	5.3
4	CIPRO	91.5 (3.7)	95.2 (1.7)	95.2 (1.7)	92.0 (3.6)	94.3 (3.0)	92.9	3.3
5	LEVO	101 (4.0)	93.5 (7.0)	93.5 (7.0)	92.5 (5.5)	102 (4.4)	95.8	6.6
6	MARBO	105 (4.8)	106 (3.1)	106 (3.1)	106 (5.7)	97.7 (5.8)	103	5.1
7	ENRO	90.1 (2.7)	91.1 (4.3)	91.1 (4.3)	93.4 (3.0)	89.5 (8.7)	91.7	4.7
8	DIFLO	87.4 (1.1)	84.2 (4.3)	84.2 (4.3)	83.9 (5.4)	80.8 (2.9)	84.1	4.7
9	PEFLO	96.7 (1.7)	87.0 (8.3)	87.0 (8.3)	82.3 (7.7)	105 (5.7)	92.5	10.2
10	OXO	118 (1.7)	109 (3.2)	109 (3.2)	105 (2.5)	103 (2.5)	108	5.9
11	FLUME	105 (4.8)	104 (7.0)	104 (7.0)	101 (5.8)	103 (2.5)	103	4.3

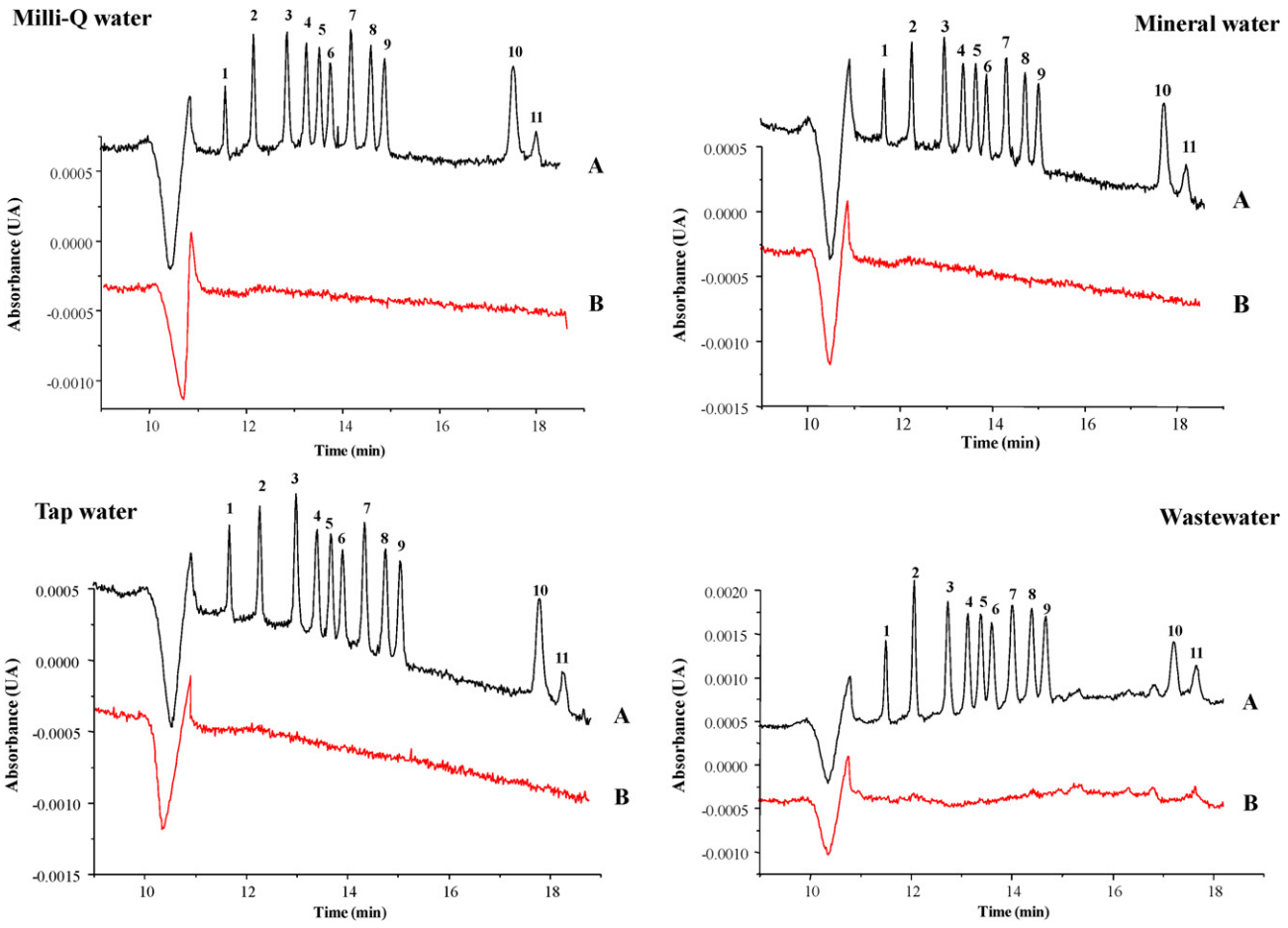

Fig. 3. LVSS-CE-DAD electropherograms of spiked water samples (Milli-Q, mineral, tap and wastewater) containing 1.0 μg/L of each antibiotic (A) and non-spiked water samples (B) after optimum o-MWCNTs-dSPE conditions (see Section 2 for details). Injection: 3.0 s at 20 psi. Sample in water containing 10% (v/v) of ACN. Separation electrolyte: 65 mM phosphate buffer at pH 8.5. Total length of the capillary: 67 cm (60 cm effective length). Voltage: +15 kV. Temperature, 25 °C. Detection at 280 nm. Peak identification: (1) MOXI, (2) LOME, (3) DANO, (4) CIPRO, (5) LEVO, (6) MARBO, (7) ENRO, (8) DIFLO, (9) PEFLO, (10) OXO and (11) FLUME.

ment (up to 20-fold) is among the ones frequently obtained for LVSS [22,58]. Comparing NSM LODs with the ones obtained with LVSS, it can be concluded that the sensitivity improvement provided by LVSS is clearly higher, a fact that has also been previously shown in the literature for the preconcentration of other analytes [55,59,60].

3.3. LVSS-CE-DAD method repeatability and calibration

Once optimum BGE composition and injection conditions were obtained, repeatability and calibration studies were developed in order to examine the performance of the LVSS-CE-DAD method. Regarding the repeatability study, it consisted in three consecutive injections of three levels of concentration (approximately 100, 250 and 400 μg/L) in the same day ($n = 3$) and in five different days ($n = 15$). Table 1 shows the results of this study for the intermediate concentration of 250 μg/L (results for the other two tested concentrations were very similar to this one). As can be seen in the table, the LVSS-CE-DAD method is highly repeatable, since RSD values in the same day were lower than 0.2% and 6.4% for migration times and peaks areas, respectively, and lower than 0.7% and 5.6% for the same parameters between different days. Calibration curves (based on the peak areas) were constructed at a working range of 38–450 μg/L by injecting each standard ($n = 8$) three times. As can be seen in Table 1, good linearity was observed with R^2 values higher than 0.996 in all cases. In addition, LOD values were between 7.19 μg/L for DANO and 16.7 μg/L for LOME, which are in the low ppb level.

3.4. Optimization of the dSPE procedure

The adsorption of CNTs can be modified by oxidation, which can remove impurities, increase the surface area as well as introduce oxygen-containing functional groups and therefore, modify the surface polarity. In particular, the introduction of carboxylic acids in the structure, although reduced since a very high oxidation creates holes on the nanotube sidewalls and significantly affects is properties [61], may introduce an additional retention mechanism: electrostatic interactions [62,63]. In this sense, fluoroquinolones, which are most of the compounds selected in our work, have a positive charge below their pK_{a2} which can be associated with carboxylate anions onto the CNT surface. Based on this consideration, we have developed a dSPE procedure using MWCNTs of 110–170 nm length in their free and oxidized (o-MWCNTs) forms as stationary phases, but first oxidation of the CNTs was carried out using HNO_3 which is the most common CNT oxidation agent [40,64,65]. Besides, refluxing with nitric acid is less vigorous and CNTs maintain their pristine electronic and mechanical properties [66]. It should also be mentioned that the presence of carboxyl groups leads to a reduction of van der Waals interactions between CNTs, which strongly facilitates the separation of nanotube bundles into individual tubes [67]. For this purpose, a previously developed procedure was adapted as described in Section 2 [40], which is based on the addition of a solution of HNO_3, sonication, heating, dilution with distilled water until neutral pH, filtration and drying under vacuum.

Table 3
Mean recoveries ($n=4$), RSD values (between parenthesis) and LODs of the selected quinolones in different water samples after the o-MWCNT-dSPE-LVSS-CE-DAD method.

Peak	Antibiotic	Milli-Q water mean recovery (RSD%)$_{n=4}$			Mineral water mean recovery (RSD%)$_{n=4}$			Tap water mean recovery (RSD%)$_{n=4}$			Wastewater mean recovery (RSD%)$_{n=4}$		
		Spiked level		LOD_{method} (μg/L)	Spiked level		LOD_{method} (μg/L)	Spiked level		LOD_{method} (μg/L)	Spiked level		LOD_{method} (μg/L)
		0.25 μg/L	1.0 μg/L		0.25 μg/L	1.0 μg/L		0.25 μg/L	1.0 μg/L		0.25 μg/L	1.0 μg/L	
1	MOXI	77.8 (2.2)	79.9 (4.7)	0.075	73.5 (1.9)	74.5 (4.2)	0.079	67.1 (3.0)	70.1 (5.0)	0.087	62.3 (5.8)	65.1 (2.9)	0.094
2	LOME	92.8 (2.4)	95.1 (2.4)	0.072	85.6 (1.7)	88.6 (1.6)	0.078	91.7 (2.9)	90.1 (7.7)	0.073	71.5 (3.3)	73.3 (2.4)	0.093
3	DANO	87.3 (2.9)	91.4 (3.0)	0.033	80.8 (2.1)	86.3 (3.1)	0.036	85.3 (4.6)	86.2 (3.9)	0.034	84.0 (3.5)	85.4 (4.6)	0.034
4	CIPRO	91.0 (4.5)	92.5 (3.7)	0.042	92.1 (3.5)	91.7 (4.3)	0.041	88.0 (3.6)	93.3 (3.3)	0.043	82.0 (2.6)	83.0 (3.5)	0.047
5	LEVO	97.0 (4.9)	101 (3.3)	0.046	89.9 (2.8)	92.0 (4.3)	0.050	94.1 (4.0)	101 (4.9)	0.047	78.5 (1.6)	80.9 (2.8)	0.057
6	MARBO	102 (0.9)	105 (4.3)	0.047	107 (4.7)	102 (3.1)	0.045	92.2 (3.5)	98.8 (5.2)	0.052	90.3 (1.9)	89.6 (2.5)	0.053
7	ENRO	88.3 (6.7)	91.2 (3.6)	0.061	90.5 (3.2)	94.0 (3.6)	0.060	82.6 (2.8)	89.9 (7.1)	0.065	67.0 (3.2)	68.5 (2.0)	0.081
8	DIFLO	88.8 (2.3)	87.3 (1.1)	0.064	83.2 (5.1)	84.2 (5.7)	0.068	82.5 (2.5)	80.8 (2.3)	0.069	73.5 (4.8)	74.5 (1.7)	0.077
9	PEFLO	93.0 (3.2)	95.6 (1.8)	0.069	83.6 (4.3)	91.5 (6.0)	0.076	96.6 (3.3)	103 (6.1)	0.066	81.8 (5.1)	82.4 (4.9)	0.078
10	OXO	114 (2.7)	116 (3.0)	0.041	106 (3.1)	108 (5.5)	0.044	102 (2.1)	103 (2.3)	0.045	94.0 (4.5)	96.9 (1.8)	0.050
11	FLUME	108 (1.8)	107 (5.0)	0.028	98.5 (2.4)	103 (3.1)	0.030	97.4 (5.3)	102 (2.1)	0.031	97.0 (2.2)	96.3 (1.1)	0.031

dSPE conditions (i.e., type and volume of the eluent, sample pH, extraction time, sample volume and amount of CNTs) were investigated and optimized in detail following a step by step approach. Preliminary experiments were developed using 25 mg of oxidized and non o-MWCNTs and 25 mL of spiked Milli-Q water at pH 5.5.

Such pH value was selected in order to ensure the protonation of the fluoroquinolones (OXO and FLUME are expected to be on their neutral form). All the experiments carried out during the optimization were developed in duplicate and considering an extraction time of 5 min. Since the polarity of the eluent is an important factor for the complete stripping of the adsorbed analytes, initially, several types of solvents of different polarities including methanol, ACN, dichloromethane and acetone, as well as these solvents with 5% (v/v) NH_4OH or formic acid (20 mL each) were compared for the selection of the optimum elution solvent. Supplementary Figure shows the results obtained in these preliminary experiments. As can be seen in the figure, acetone and methanol showed the highest extraction recoveries for all the analytes. In particular, acetone yielded relatively high recovery percentages for the peaks 4–11, while peaks 1, 2 and 3 had the highest recovery percentages with the use of methanol. On the contrary, dichloromethane was the solvent with the lowest efficiency of all. When the behavior of o-MWCNTs and non oxidized MWCNTs was compared, it is clear that o-MWCNTs showed a higher extraction capacity for all the studied analytes and elution solvents. Concerning OXO and FLUME such differences are in general not so remarked since these compounds are not positively charged at the studied pH. Experiments developed with the addition of formic acid or ammonia to the elution solvent clearly decreased the extraction recoveries in all cases. As a result of these experiments, we decided to study the elution with mixtures of acetone and methanol (3:1, 1:1 and 1:3, v/v). Among them, the mixture 3:1 (v/v) acetone/methanol provided the highest recovery values in all cases. As a result, this eluent, as well as o-MWCNTs, was used in subsequent experiments.

As previously indicated, according to their chemical structure, the charge of the studied quinolones, as well as the o-MWCNT surface, can be affected by the pH of the sample, therefore, their retention onto the o-MWCNTs could be influenced by this variable. In this work, the sample pH was investigated in the range 4.0–6.5 by adding appropriate volumes of 0.1 M HCl or 0.1 M NaOH. Fig. 2 shows the influence of sample pH on the extraction of the selected compounds. According to these results, for most antibiotics the recoveries increased when increasing pH. Above pH 5.0 recovery percentages decrease. Thus, pH 5.0 was selected since it yielded recovery percentages in the range 72.3–114% for all the analytes, except for MOXI, which was 63.6%.

Once the elution solvent and the sample pH were studied, the amount of o-MWCNTs and sample volume were investigated in order to use the lowest amount of sorbent and to improve the sensitivity of the whole method as possible without decreasing recovery percentages. For this purpose, extraction of 20–250 mL of spiked Milli-Q sample was developed in duplicate, using 100, 150 and 200 mg of o-MWCNTs. Experimental results demonstrated that there were no significant differences in the enrichment efficiencies of the analytes by using 150 and 200 mg of sorbent (the use of 100 mg of o-MWCNT resulted in lower recoveries), even with sample volumes of 250 mL. The use of these conditions (150 mg of o-MWCNTs and 250 mL of Milli-Q at pH 5.0) gave the highest mean recovery values (in the range 64–114%). However, an increase in the sorbent amount affects not only the quantity of analyte that can be retained, but also the volume of eluent required. Thus, elution was carried out using 20, 25, 30, 35 and 40 mL of 3:1 (v/v) acetone/methanol. Fig. 2 also shows the results of these experiments in which it can clearly be seen that a volume of 25 mL was sufficient to desorb the trapped quinolones from

Table 4
Results of assays to check the precision and accuracy of the proposed method for the selected quinolones in water samples.

Antibiotic	Spiked level (μg/L)	Water	Found (μg/L)[a]	Accuracy (%)	t_{exp}[b]	Antibiotic	Spiked level (μg/L)	Water	Found (μg/L)[a]	Accuracy (%)	t_{exp}[b]
MOXI	0.392	Milli-Q	0.396 ± 0.022	101	0.34	ENRO	0.398	Milli-Q	0.395 ± 0.027	99	0.36
		Mineral	0.388 ± 0.012	99	1.41			Mineral	0.384 ± 0.020	96	0.79
		Tap	0.400 ± 0.025	102	0.69			Tap	0.408 ± 0.024	103	3.54
		Waste	0.380 ± 0.031	97	2.19			Waste	0.411 ± 0.044	103	1.28
	0.784	Milli-Q	0.776 ± 0.024	99	0.74		0.797	Milli-Q	0.808 ± 0.025	101	2.11
		Mineral	0.800 ± 0.012	102	0.80			Mineral	0.786 ± 0.018	99	0.16
		Tap	0.776 ± 0.025	99	1.39			Tap	0.811 ± 0.022	102	0.62
		Waste	0.768 ± 0.032	98	2.77			Waste	0.775 ± 0.041	97	1.01
LOME	0.384	Milli-Q	0.392 ± 0.035	102	1.04	DIFLO	0.375	Milli-Q	0.376 ± 0.041	100	0.02
		Mineral	0.380 ± 0.047	99	0.49			Mineral	0.370 ± 0.033	99	0.17
		Tap	0.399 ± 0.014	104	2.42			Tap	0.388 ± 0.044	103	2.10
		Waste	0.372 ± 0.043	97	1.73			Waste	0.390 ± 0.034	104	2.51
	0.768	Milli-Q	0.753 ± 0.037	98	1.22		0.750	Milli-Q	0.745 ± 0.043	99	0.20
		Mineral	0.760 ± 0.049	99	2.16			Mineral	0.761 ± 0.034	101	0.78
		Tap	0.776 ± 0.015	101	0.99			Tap	0.775 ± 0.046	103	1.94
		Waste	0.783 ± 0.045	102	2.08			Waste	0.739 ± 0.025	99	0.12
DANO	0.320	Milli-Q	0.311 ± 0.011	97	0.39	PEFLO	0.400	Milli-Q	0.403 ± 0.019	101	0.69
		Mineral	0.329 ± 0.031	103	2.42			Mineral	0.395 ± 0.034	99	0.33
		Tap	0.334 ± 0.014	104	2.54			Tap	0.383 ± 0.042	96	0.97
		Waste	0.311 ± 0.019	97	3.78			Waste	0.377 ± 0.021	94	3.32
	0.639	Milli-Q	0.630 ± 0.011	99	1.29		0.800	Milli-Q	0.796 ± 0.017	100	0.09
		Mineral	0.651 ± 0.032	102	1.71			Mineral	0.804 ± 0.020	101	0.58
		Tap	0.642 ± 0.015	100	0.65			Tap	0.793 ± 0.011	99	1.19
		Waste	0.621 ± 0.020	97	0.92			Waste	0.789 ± 0.043	99	0.24
CIPRO	0.335	Milli-Q	0.336 ± 0.016	100	0.09	OXO	0.404	Milli-Q	0.400 ± 0.019	99	0.38
		Mineral	0.340 ± 0.016	101	0.26			Mineral	0.410 ± 0.012	101	1.16
		Tap	0.321 ± 0.013	96	0.92			Tap	0.398 ± 0.022	99	0.70
		Waste	0.327 ± 0.036	98	2.04			Waste	0.386 ± 0.034	96	1.72
	0.671	Milli-Q	0.676 ± 0.017	101	0.68		0.807	Milli-Q	0.811 ± 0.041	100	0.10
		Mineral	0.652 ± 0.016	97	0.87			Mineral	0.800 ± 0.039	99	0.33
		Tap	0.684 ± 0.013	102	1.53			Tap	0.796 ± 0.010	99	0.51
		Waste	0.650 ± 0.038	97	3.19			Waste	0.789 ± 0.015	98	3.80
LEVO	0.384	Milli-Q	0.379 ± 0.026	99	0.17	FLUME	0.398	Milli-Q	0.398 ± 0.027	100	0.01
		Mineral	0.383 ± 0.015	100	0.02			Mineral	0.379 ± 0.016	95	2.09
		Tap	0.379 ± 0.018	99	1.63			Tap	0.407 ± 0.044	102	2.77
		Waste	0.381 ± 0.023	99	0.98			Waste	0.395 ± 0.037	99	0.25
	0.768	Milli-Q	0.766 ± 0.027	100	0.08		0.795	Milli-Q	0.800 ± 0.028	101	3.84
		Mineral	0.763 ± 0.016	99	0.74			Mineral	0.787 ± 0.018	99	0.17
		Tap	0.765 ± 0.018	100	0.06			Tap	0.809 ± 0.026	102	1.16
		Waste	0.760 ± 0.024	99	2.01			Waste	0.777 ± 0.030	98	0.44
MARBO	0.379	Milli-Q	0.384 ± 0.041	101	1.22						
		Mineral	0.368 ± 0.022	97	0.49						
		Tap	0.359 ± 0.042	95	1.98						
		Waste	0.336 ± 0.031	89	2.44						
	0.759	Milli-Q	0.762 ± 0.042	100	0.10						
		Mineral	0.775 ± 0.023	102	1.27						
		Tap	0.733 ± 0.030	97	0.84						
		Waste	0.721 ± 0.027	95	1.05						

t: experimental *t* value.

[a] Average value ± standard deviation of 3 determinations (95% confidence level).

[b] $t_{tab} = 4.30$, $\alpha = 0.05$.

the o-MWCNTs (higher volumes provided similar recovery values).

Extraction time was also studied between 0 and 30 min. No significant differences in the recovery values were observed for any of the selected quinolones and, as a result, it was not necessary to maintain it constant at a given value.

Therefore, optimum dSPE conditions were the following: 250 mL of water at pH 5.0, 150 mg of o-MWCNTs and 25 mL of 3:1 (v/v) acetone/methanol. Under these conditions, mean recovery values were between 79% (for MOXI) and 116% (for OXO), showing the potential of the o-MWCNTs-dSPE-LVSS-CE-DAD method for the analysis of these eleven quinolones at trace levels in water samples. Moreover, with the aim of comparing these results with those obtained using the same MWCNTs without oxidation, the same whole method under optimum conditions was applied. Results showed that mean recovery percentages by using the o-MWCNTs (77.8–116%) were higher than those obtained using MWNCTs (49.3–74.5%, except for OXO and FLUME which were around 100%), demonstrating the high extraction capacity of the o-MWCNTs.

In order to reveal the repeatability of the oxidation procedure, three consecutive extractions of spiked Milli-Q water samples at 1.0 μg/L was carried out with o-MWCNTs obtained from five different oxidations (they were carried out in different days). Results of these assays (mean recovery and RSD values) are shown in Table 2. As can be seen, there is a good repeatability between oxidations which clearly demonstrates the robustness of the procedure.

3.5. Recovery study and real sample analysis

The whole method was then applied to the analysis of Milli-Q, mineral, tap and wastewater samples in order to demonstrate its. For this purpose, a recovery study was firstly carried out at two concentration levels (0.25 and 1.0 μg/L) which were extracted four times each ($n = 4$) and injected in triplicate in the CE system following the optimized LVSS method. The wastewater sample was collected at a wastewater treatment plant at the south of Tenerife, which corresponds to a tertiary treatment. Preliminary analysis of non-spiked samples showed that they were free of the selected antibiotics and that no interferences which overlapped with the target analytes appeared. Table 3 shows mean recoveries and RSD values of spiking each level for the four different types of water. As can be seen in the table, mean recoveries were in the range 77.8–116% for Milli-Q water (with RSD < 6.7%), 73.5–108% for mineral water (RSD < 6.0%), 67.1–103% for tap water (RSD < 7.7%) and 62.3–97.0% for wastewater (RSD < 5.8%). According to these values, LODs of the o-MWCNTs-dSPE-LVSS-CE-DAD method (see Table 3) range 28–75 ng/L for Milli-Q water, 30–79 ng/L for mineral water, 31–87 ng/L for tap water and 31–94 ng/L for wastewater. These data were experimentally checked by the extraction of samples spiked at the LOD level and calculation of the S/N. It is interesting to mention that these sensitive LODs as well as the recoveries obtained are comparable to the ones obtained in previous works for the analysis of quinolones in different water samples by SPE-CE [17,19] and even by SPE-HPLC [68–70] using conventional SPE cartridges. However, the use of the dSPE procedure described in this work involves a highly selective and a more simple procedure that can be easily applied to different water matrices.

All these results revealed that the proposed methodology is very sensitive, repeatable, selective, with high extractability capacity and that it can be used for the ultrapreconcentration of the quinolones from different water samples. Fig. 3 shows the electropherograms of a spiked (A) and a non-spiked (B) Milli-Q, mineral, tap and wastewater samples after the application of the optimum o-MWCNT-dSPE-CE-DAD method. As can be seen from the figures, no interferences from the sample matrix overlapped the target analytes, not even for wastewater, despite the fact that it is a very complex sample obtained from a treatment plant and with high amounts of organic matter.

Finally, with the aim of verifying the precision and the accuracy of the developed method, a statistical comparison (Student's t-test) was developed. For this purpose, three consecutive extractions of spiked water samples at two concentration levels were carried out. Table 4 shows the results of this study, which revealed that experimental t values for all the quinolones ($t \leq 3.84$) were lower than the tabulated one (4.30 for $n = 3$, $\alpha = 0.05$), which means that there are no significant differences between the real and the found concentration (the null hypothesis can be accepted). In addition, accuracy percentages ranged between 89 and 104%.

4. Conclusions

In this work, MWCNTs were functionalized (oxidized) and used for the first time as stationary phases for the simultaneous dSPE of eleven quinolones from different water samples (Milli-Q, mineral, tap and wastewater) using CE-DAD as separation technique. LVSS was used as on-line preconcentration method, providing an overall sensitivity of the method of 28–94 ng/L. The proposed procedure is simple, selective, repeatable and effective, as demonstrated by the recovery and precision and accuracy studies carried out.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.06.031.

References

[1] W.W. Buchberger, J. Chromatogr. A 1218 (2011) 603.
[2] V. Andreu, C. Blasco, Y. Picó, Trends Anal. Chem. 26 (2007) 534.
[3] N. Le-Minh, S.J. Khan, J.E. Drewes, R.M. Stuetz, Water Res. 44 (2010) 4295.
[4] A. Speltini, M. Sturini, F. Maraschi, A. Profumo, J. Sep. Sci. 33 (2010) 1115.
[5] S. Mompelat, B. Le Bot, O. Thomas, Environ. Int. 35 (2009) 803.
[6] P.A. Segura, M. François, C. Gagnon, S. Sauvé, Environ. Health Perspect. 117 (2009) 675.
[7] K. Kümmerer, Chemosphere 75 (2009) 417.
[8] N. Kemper, Ecol. Ind. 8 (2008) 1.
[9] M.D. Prat, J. Benito, R. Compañó, J.A. Hernández-Arteseros, M. Granados, J. Chromatogr. A 1041 (2004) 27.
[10] H. Nakata, K. Kannan, P.D. Jones, J.P. Giesy, Chemosphere 58 (2005) 759.
[11] H.B. Lee, T.E. Peart, M.L. Svoboda, J. Chromatogr. A 1139 (2007) 45.
[12] M. Seifrtová, A. Pena, C.M. Lino, P. Solich, Anal. Bioanal. Chem. 391 (2008) 799.
[13] A. Pena, D. Chmielova, C.M. Lino, P. Solich, J. Sep. Sci. 30 (2007) 2924.
[14] A.V. Herrera-Herrera, J. Hernández-Borges, M.A. Rodríguez-Delgado, Anal. Bioanal. Chem. 292 (2008) 1439.
[15] M. Seifrtová, L. Nováková, C. Lino, A. Pena, P. Solich, Anal. Chim. Acta 649 (2009) 158.
[16] E. Turiel, G. Bordin, A.R. Rodríguez, J. Chromatogr. A 1008 (2003) 145.
[17] M. Ferdig, A. Kaleta, T.D.T. Vo, W. Buchberger, J. Chromatogr. A 1047 (2004) 305.
[18] A.V. Herrera-Herrera, J. Hernández-Borges, T.M. Borges-Miquel, M.A. Rodríguez-Delgado, Electrophoresis 31 (2010) 3457.
[19] M. Lombardo-Agüí, L. Gámiz-Gracia, A.M. García-Campaña, C. Cruces-Blanco, Anal. Bioanal. Chem. 396 (2010) 1551.
[20] J.P. Quirino, S. Terabe, J. Chromatogr. A 902 (2000) 119.
[21] M.C. Breadmore, M. Dawod, J.P. Quirino, Electrophoresis 32 (2011) 127.
[22] S.L. Simpson Jr., J.P. Quirino, S. Terabe, J. Chromatogr. A 1184 (2008) 504.
[23] H.-B. He, X.-X. Lv, Q.-W. Yu, Y.-Q. Fen, Talanta 82 (2010) 1562.

[24] K.D. Altria, Y.L. Chanter, J. Chromatogr. A 652 (1993) 459.
[25] M. Hernández, F. Borrull, M. Calull, Chromatographia 55 (2002) 585.
[26] M. Hernández, C. Aguilar, F. Borrull, M. Calull, J. Chromatogr. B 772 (2002) 163.
[27] R.-L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 1046.
[28] M. Valcárcel, S. Cárdenas, B.M. Simonet, Anal. Chem. 79 (2007) 4788.
[29] L.M. Ravelo-Pérez, A.V. Herrera-Herrera, J. Hernández-Borges, M.A. Rodríguez-Delgado, J. Chromatogr. A 1217 (2010) 2618.
[30] M. Asensio-Ramos, J. Hernández-Borges, L.M. Ravelo-Pérez, M.A. Rodríguez-Delgado, Electrophoresis 29 (2008) 4412.
[31] B. Suárez, B. Santos, B.M. Simonet, S. Cárdenas, M. Valcárcel, J. Chromatogr. A 1175 (2007) 127.
[32] H. Niu, Y. Cai, Y. Shi, F. Wei, J. Liu, S. Mou, G. Jiang, Anal. Chim. Acta 594 (2007) 81.
[33] G.-Z. Fang, J.-X. He, S. Wang, J. Chromatogr. A 1127 (2006) 12.
[34] Y. Lu, Q. Shen, Z. Dai, H. Zhang, Anal. Bioanal. Chem. 398 (2010) 1819.
[35] G. Morales-Cid, A. Fekete, B.M. Simonet, R. Lehmann, S. Cárdenas, X. Zhang, M. Valcárcel, P. Schmitt-Kopplin, Anal. Chem. 82 (2010) 2743.
[36] W.-H. Tsai, T.-C. Huang, J.-J. Huang, Y.H. Hsue, H.Y. Chuang, J. Chromatogr. A 1216 (2009) 2263.
[37] Q. Lu, X. Chen, L. Nie, H. Jiang, L. Chen, Q. Hu, S. Du, Z. Zhang, Talanta 81 (2010) 959.
[38] Y. Peng, Y. Xie, J. Luo, L. Nie, Y. Chen, L. Chen, S. Du, Z. Zhang, Anal. Chim. Acta 674 (2010) 190.
[39] M. Asensio-Ramos, G. D'Orazio, J. Hernández-Borges, S. Fanali, Anal. Bioanal. Chem. 400 (2011) 1113.
[40] M.J. Park, J.K. Lee, B.S. Lee, Y.W. Lee, I.S. Choi, S. Lee, Chem. Mater. 18 (2006) 1546.
[41] J. Barbosa, R. Bergés, I. Toro, N.V. Sanz, Talanta 49 (1997) 1271.
[42] J. Barbosa, G. Fonrodona, I. Marqués, S. Buti, I. Toro, Trends Anal. Chem. 16 (1997) 104.
[43] N.K. Takács, B. Noszál, G. Keresztúri, B. Podányi, G. Szász, J. Pharm. Sci. 79 (1990) 1023.
[44] E. Jiménez-Lozano, I. Marqués, D. Barrón, J.L. Beltrán, J. Barbosa, Anal. Chim. Acta 464 (2002) 37.
[45] B. Sandra, J.M.H. Alka, M.P. Dragana, K.M. Marija, Trends Anal. Chem. 26 (2007) 1043.
[46] C.-E. Lin, Y.-J. Deng, W.-S. Liao, S.-W. Sun, W.-Y. Lin, C.-C. Chen, J. Chromatogr. A 1051 (2004) 283.
[47] L.W. Zhang, K. Wang, X.X. Zhang, Anal. Chim. Acta 603 (2007) 101.
[48] A. Juan-García, G. Font, Y. Picó, Electrophoresis 27 (2006) 2240.
[49] C. Fierens, S. Hiollaert, W. Van-den-Bossche, J. Pharm. Biomed. Anal. 22 (2000) 763.
[50] M. Hernández, F. Borrull, M. Calull, J. Chromatogr. B 742 (2000) 255.
[51] J.L. Beltrán, E. Jiménez-Lozano, D. Barrón, J. Barbosa, Anal. Chim. Acta 501 (2004) 137.
[52] D. Barrón, A. Irles, J. Barbosa, J. Chromatogr. A 871 (2000) 367.
[53] S.Y. Chang, F.Y. Wang, J. Chromatogr. B 799 (2004) 265.
[54] J. Hernández-Borges, M.A. Rodríguez-Delgado, F.J. García-Montelongo, A. Cifuentes, J. Sep. Sci. 28 (2005) 948.
[55] L.M. Ravelo-Pérez, J. Hernández-Borges, A. Cifuentes, M.A. Rodríguez-Delgado, Electrophoresis 28 (2007) 1805.
[56] Z.K. Shiabi, J. Chromatogr. A 1066 (2005) 205.
[57] Z.K. Shiabi, J. Chromatogr. A 817 (1998) 25.
[58] D.M. Osbourn, D.J. Wiss, C.E. Lunte, Electrophoresis 14 (2000) 2768.
[59] J. Hernández-Borges, A. Cifuentes, F.J. García-Montelongo, M.A. Rodríguez-Delgado, Electrophoresis 26 (2005) 980.
[60] J. Hernández-Borges, F.J. García-Montelongo, A. Cifuentes, M.A. Rodríguez-Delgado, J. Chromatogr. A 1070 (2005) 171.
[61] S. Wang, Curr. Appl. Phys. 9 (2009) 1146.
[62] K. Shrivas, H.F. Wu, Anal. Chim. Acta 628 (2008) 198.
[63] Y. Ji, X. Liu, X. Jiang, H. Huang, Z. Xu, H. Zhang, C. Wang, Chromatographia 70 (2009) 753.
[64] I.D. Rosca, F. Watari, M. Uo, T. Akasaka, Carbon 43 (2005) 3124.
[65] Y.C. Chiang, W.H. Lin, Y.C. Chang, Appl. Surf. Sci. 257 (2011) 2401.
[66] J. Zhang, H. Zou, Q. Qing, Y. Yang, Q. Li, Z. Liu, X. Guo, Z. Du, J. Phys. Chem. B 107 (2003) 3712.
[67] K. Balasubramanian, M. Burghard, Small 1 (2005) 180.
[68] E.M. Golet, A.C. Alder, A. Hartmann, T.A. Ternes, W. Giger, Anal. Chem. 73 (2001) 3632.
[69] M. Ferdig, A. Kaleta, W. Buchberger, J. Sep. Sci. 28 (2005) 1448.
[70] J.E. Renew, C.H. Huang, J. Chromatogr. A 1042 (2004) 113.