Talanta 230 (2021) 122307

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of chloramphenicol and tetracycline residues in milk samples by means of nanofiber coated magnetic particles prior to high-performance liquid chromatography-diode array detection

Busra Vuran [a], Halil Ibrahim Ulusoy [a], Gokhan Sarp [b,c], Erkan Yilmaz [b,c,d], Ummügülsüm Morgül [a], Abuzar Kabir [e], Angela Tartaglia [f], Marcello Locatelli [f], Mustafa Soylak [d,g,h,*]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Cumhuriyet University, Sivas, Turkey
[b] Department of Analytical Chemistry, Faculty of Pharmacy, Erciyes University, Kayseri, Turkey
[c] ERNAM Erciyes University, Nanotechnology Application and Research Center, 38039, Kayseri, Turkey
[d] Technology Research and Application Center (TAUM), Erciyes University, 38039, Kayseri, Turkey
[e] Department of Chemistry and Biochemistry, Florida International University, 11200 SW 8th St, Miami, FL, 33199, USA
[f] Department of Pharmacy, University of Chieti–Pescara "G. d'Annunzio", Via dei Vestini 31, Chieti, 66100, Italy
[g] Department of Chemistry, Faculty of Sciences, Erciyes University, Kayseri, Turkey
[h] Turkish Academy of Sciences (TUBA), Cankaya, Ankara, Turkey

ARTICLE INFO

Keywords:
Chloramphenicol
Tetracycline
HPLC
Carbon nanofiber
Magnetic solid phase extraction
Milk samples

ABSTRACT

A magnetic solid phase extraction (MSPE) coupled with high-performance liquid chromatography-diode array detection (HPLC-DAD) methodology was developed for the determination of chloramphenicol (CP) and tetracycline (TET) antibiotic residues in milk samples. As a solid phase sorbent, C-nanofiber coated magnetic nanoparticles were synthesized and extensively characterized using Field Emission Scanning Electron Microscopy (FE-SEM), Raman Spectroscopy and X-ray Powder Diffraction (XRD) analysis. Experimental variables of MSPE method for both antibiotic analytes were investigated and optimized systematically. After MSPE, the linear range for both the analytes ($r^2 > 0.9954$) were obtained in a range 10.0–600.0 ng mL^{-1}. The limit of detections (LODs) for CP and TET were 3.02 and 3.52 ng mL^{-1}, respectively while RSDs % were below than 4.0%. Finally, the developed method based on MPSE-HPLC-DAD was applied to real milk samples to quantify the antibiotic residues. Recovery values for each antibiotic compound were found in the range of 94.6–105.4% (n = 3) by using spiked model solution.

1. Introduction

Tetracyclines, a group of antibiotics, are one of the main antibiotic groups used for veterinary, human therapy and agricultural purposes and tetracycline is one of these antibiotics. Tetracycline, naturally produced by the bacterium "*Streptomyces rimosus*" belongs to tetracyclines antibiotic family and is used in the management and treatment of a variety of infectious diseases [1]. Tetracycline is effective in a broad spectrum of microbial in the body, such as gram (+) bacteria, gram (−) bacteria, rickettsia, chlamydia, mycoplasmas and amoebas. Although it is considered as a broad spectrum antibiotic, it is known as one of the least selective antibiotics [2,3]. Chloramphenicol is an antibiotic that was first obtained as the metabolism product of bacteria of the "*Streptomyces venezuelae*" type, and can now be obtained artificially [4]. It is effective against many pathogenic bacteria, rickettsia and mycoplasma and shows this effect by disrupting the protein synthesis in microorganisms [5]. Chloramphenicol is not a highly preferred antibiotic to use due to its many side effects. In very special cases, it is used in the treatment of deadly infections such as cholera, typhoid and fever. Chloramphenicol is used to kill vibrios that are particularly resistant to tetracycline. For this reason, chloramphenicol and tetracycline derivative antibiotics are used together for treatment purposes [6].

However, excessive amounts of these antibiotics not only cause the widespread diffusion of antibiotic in water and instigate environmental

* Corresponding author. Technology Research and Application Center (TAUM), Erciyes University, 38039, Kayseri, Turkey.
E-mail address: soylak@erciyes.edu.tr (M. Soylak).

https://doi.org/10.1016/j.talanta.2021.122307
Received 27 November 2020; Received in revised form 5 March 2021; Accepted 8 March 2021
Available online 16 March 2021

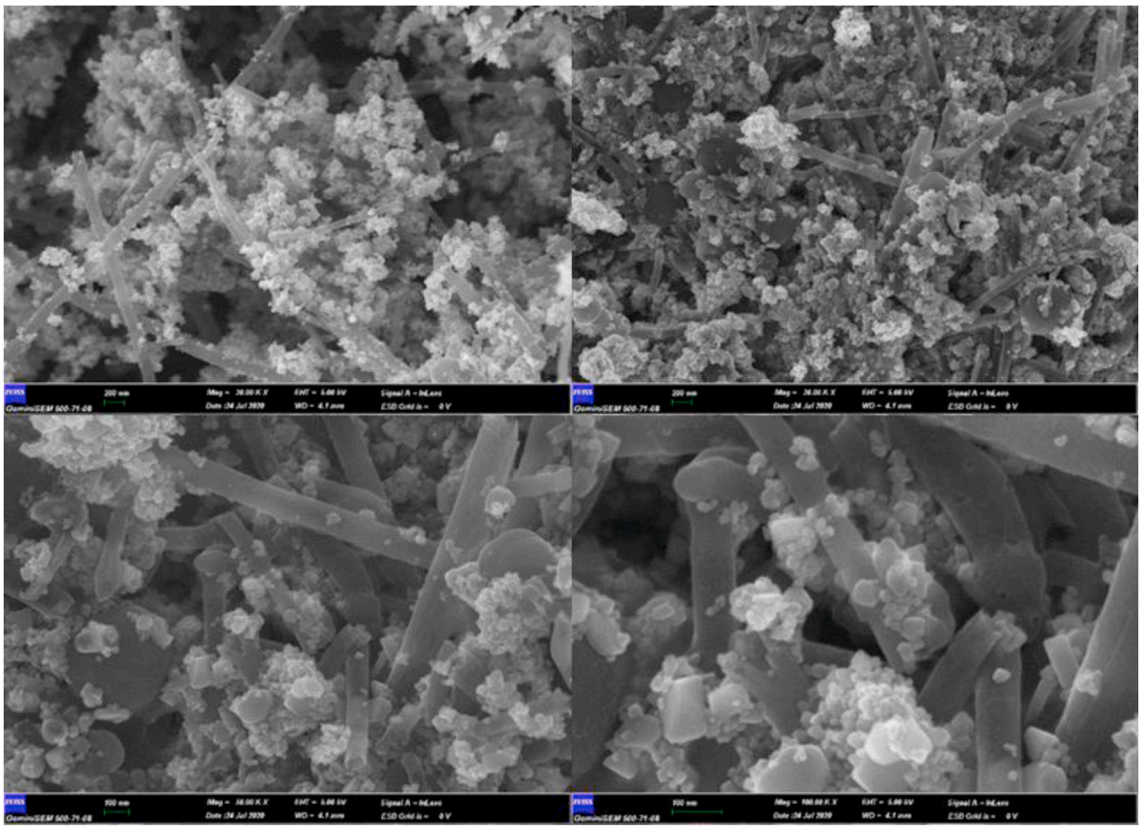

Fig. 1. FESEM images of C-nanofiber coated magnetic nanoparticles.

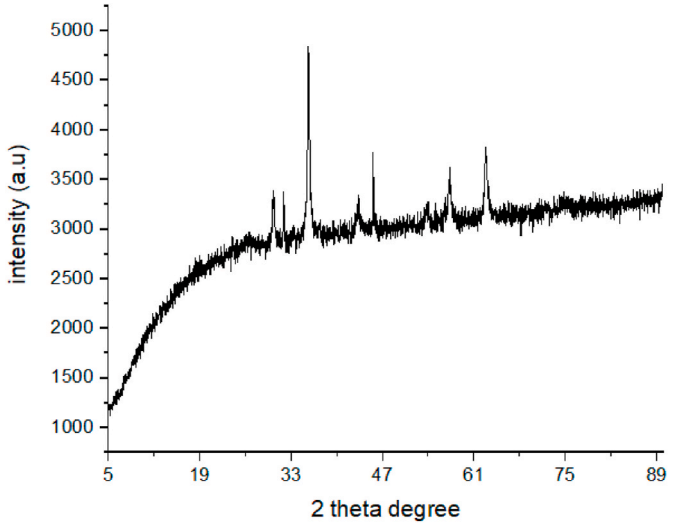

Fig. 2. XRD spectrum of C-nanofiber coated magnetic nanoparticles.

pollution, but also accumulate in animal tissues and edible animal products such as beef, mutton, pork, chicken, milk [7,8].

The methods available in the literature for the determination of chloramphenicol and tetracycline include high performance liquid chromatography (HPLC) [9], liquid chromatography-mass spectrometry (LC-MS) [10], molecular spectroscopy [11], spectrofluorimetric [12], gas chromatography (GC) [13], gas chromatography-mass spectrometry (GC-MS) [14]. However, due to the trace levels in complex food matrices and the disruptive effects of matrix components, pre-separation and pre-concentration techniques have become an inevitable stage prior to analysis of these antibiotics [15].

It is of great importance to develop a new sample preparation technique, which is inherently simple, fast, efficient, inexpensive, possesses high analyte retention capacity and allows regeneration for using same sorbent multiple times [16–18]. SPE offers significant advantages over conventional liquid-liquid extraction (LLE) methods in terms of high analyte recovery, highly purified extracts, the ability to simultaneously extract a wide range of polarity analytes and reduce organic solvent [19, 20]. However, the disadvantages of the cartridge configuration of traditional SPE techniques in which the adsorbents are filled, such as the time-consuming steps, the use of excessive amount of solvent, and the large amount of waste after the process, limit their use.

Magnetic solid phase extraction (MSPE), a new SPE approach, is performed based on its ability to adsorb and desorb analytes on a magnetic adsorbent ranging from mg or μg using an external magnetic field without some tedious steps (centrifugation or filtration). Sorbent particles can be easily isolated and collected during adsorption and desorption, making the sample pre-treatment procedure more convenient, time-saving and cost-effective [21–23]. When magnetic solid phase extraction methods in the literature are examined, it is seen that carbon-based nanoparticles such as carbon nanotubes (CNTs), fullerene, nano diamond and carbon nanofibers (C-NFs), which have high surface area and adsorption capacity and can be used repeatedly due to their inertness in the working solution environments, are frequently preferred as adsorbents [24–27].

In this work, C-nanofiber coated magnetic nanoparticles were synthesized and used as sorbent in a magnetic solid phase extraction of tetracycline and chloramphenicol at trace levels from milk samples. Together with the HPLC-DAD, the main analytical factors that affected the extraction efficiency in the experiment were carefully optimized. The method was validated by standard addition and recovery experiments. The satisfactory material performance of the adsorbent showed that it could be used as a promising material in the development of MSPE techniques with high analytical performance.

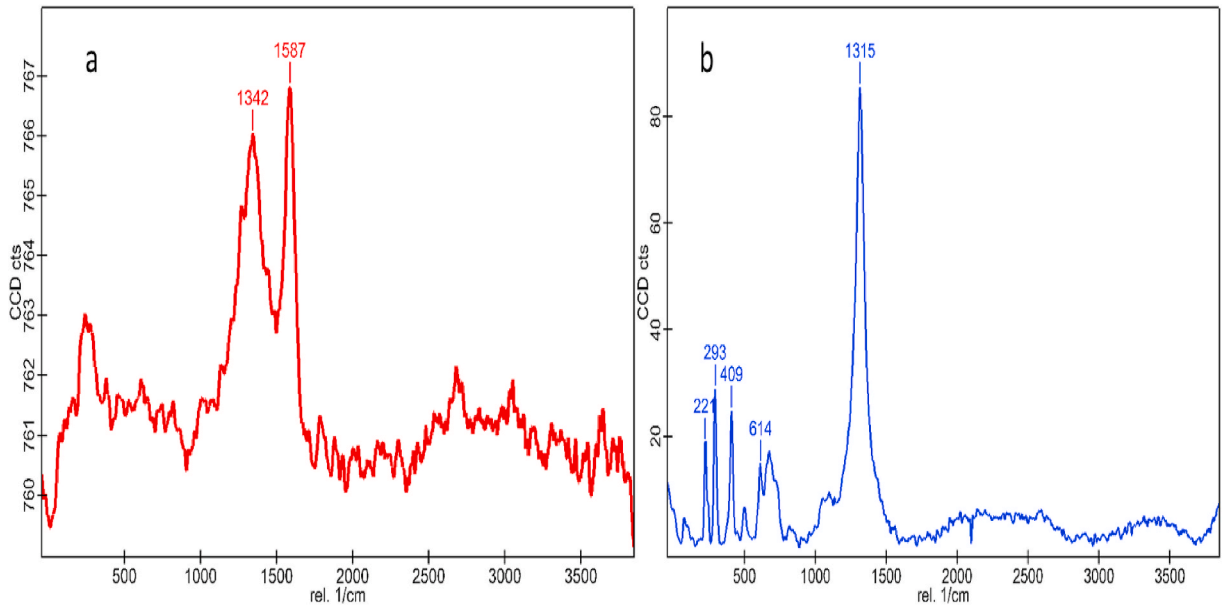

Fig. 3. a. Raman spectrum of C-nanofiber coated magnetic nanoparticles, b. Raman spectrum of Fe_3O_4 nanoparticles.

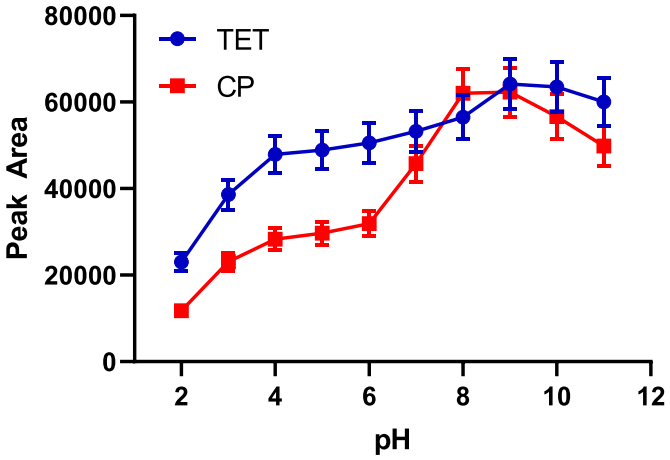

Fig. 4. The effect of pH on extraction efficiency of TET and CP (N = 3).

2. Materials and methods

2.1. Instrumentation

Characterization of synthesized magnetic C-nanofibers was carried out by using Field Emission Scanning Electron Microscopy (FE-SEM), Raman Spectroscopy and X-ray Powder Diffraction (XRD) techniques. The Raman spectra of the nanomaterials were obtained using a Raman Spectrophotometer (WITec alpha 300 M + micro-Raman system, Germany) with a 532 nm laser source. X-ray diffraction spectrum of magnetic C-nanofibers was taken with a Bruker AXS D8 brand X-ray diffractometer. SEM and SEM-Mapping analysis were performed using a Zeiss Gemini 500 Field Emission Scanning Electron Microscope to elucidate the morphological structures of C-nanofibers and magnetic C-nanofibers. Chromatographic analysis of Tetracycline and Chloramphenicol were performed by the Shimadzu (Prominence) HPLC (Kyoto, Japan) system. All separations and determinations were done on a phenyl hexyl column (Luna® 5 µm Phenyl-Hexyl 100 Å, 250 mm × 4.6 mm). All mobile phases required for analysis were prepared for use in HPLC-DAD analysis using HPLC grade acetonitrile and methanol (Sigma Aldrich, St. Louis, MO, USA).

2.2. Chemicals and reagents

In this study, all chemicals used are at the rate of 99.5% purity. Deionized water system had 18.2 MΩ cm resistivity was used to obtain deionized water (MES, MP Minipure Dest Up, Turkey). The HPLC grade acetonitrile (ACN) and methanol (MeOH) were used for HPLC-DAD analysis (Sigma Aldrich, St. Louis, MO, USA). For HPLC analysis, a mixture of phosphate buffer solution (pH 6.0, 50 mM), methanol and acetonitrile (60:10:30) was used as the mobile phase. A stock solution of Tetracycline and Chloramphenicol had 100 µg mL^{-1} concentration was prepared in the analytical purity of methanol (Sigma Aldrich, St. Louis, MO, USA). In the synthesis of magnetic C-nanofibers, C-nanofibers with 100 nm in diameter and 20–200 µm in length, $FeCl_3$, $NaCH_3COO$ and ethylene glycol were used (Sigma Aldrich, ST Louis, USA).

2.3. Synthesis of C-nanofiber coated magnetic nanoparticles

C-nanofiber coated magnetic nanoparticles were produced by using hydrothermal synthesis method in a solvothermal production reactor. For this purpose, 1.0 g of iron (III) chloride and 4.0 g of sodium acetate were dissolved in a solution containing 25 mL of deionized water and 50 mL of ethylene glycol. 0.50 g C-nanofibers was homogeneously dispersed in this solution by applying ultrasonic vibration for 2 h in an ultrasonic water bath. The resulting mixture was allowed to react in the reactor at 180 °C for 12 h. The C-nanofiber coated magnetic nanoparticles obtained after the completion of the hydrothermal reaction process were collected with an external neodymium magnet, then washed several times with deionized water and ethanol and dried in a vacuum oven.

2.4. Magnetic solid phase extraction

Magnetic solid phase extraction optimization and real sample studies were carried out in 50 mL centrifuge tubes. For this, 3 mL of pH 9 buffer solutions were added to 5 mL sample or model solutions containing antibiotic molecules and the final volumes were completed to 50 mL with deionized water. 50 mg of magnetic C-nanofiber was added to these solutions and the caps of the tubes were tightly closed and placed in a shaker device. Stirring was applied to the tubes at 80 rpm for 30 min. After the set time expired, a neodymium magnet was held from the outer part of the tubes and the adsorbent particles with magnetic properties

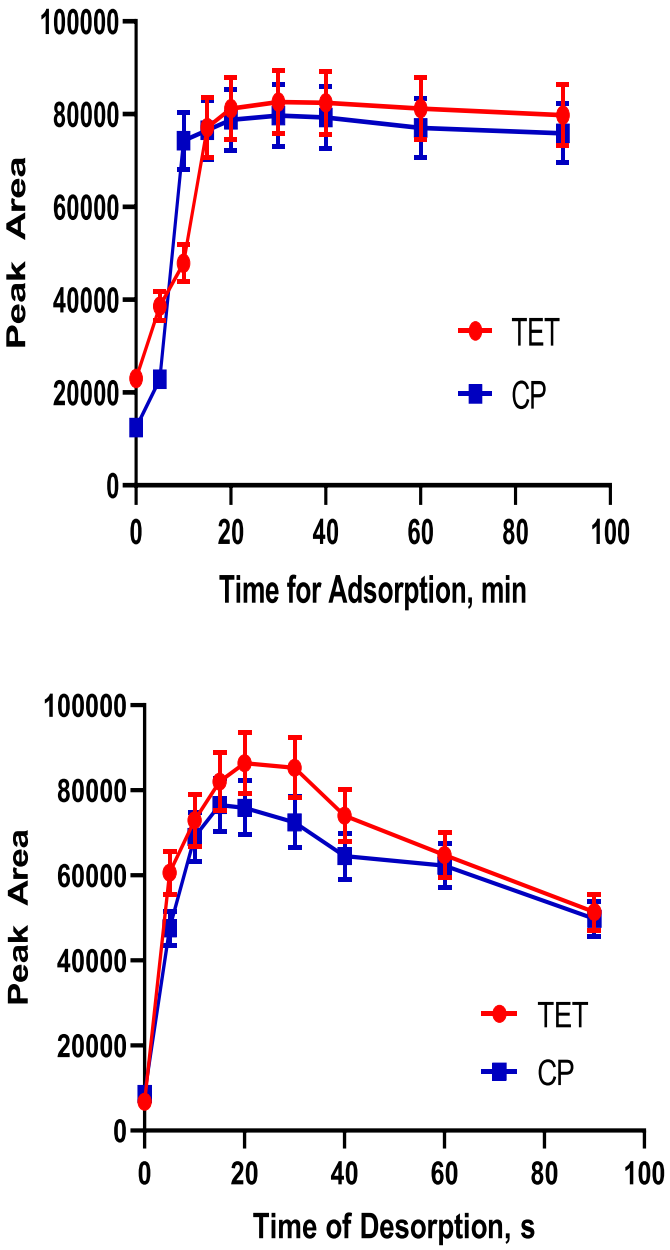

Fig. 5. a) The effect of adsorption time on adsorption equilibrium of TET and CP (N = 3) b) The effect of vortex duration time on desorption efficiency of TET and CP (N = 3).

were collected within the wall of the tube. The aqueous phases were decanted and removed from the adsorbent. After the aqueous phases were removed, 500 μL of ACN: MeOH (1:1) was added on the solid phase and the tubes were vortexed for 40 s. With this process, the analyte was desorbed from the adsorbent. The eluent phases were separated from the adsorbent phase by holding a neodymium magnet from the outer part of the tubes. The eluents were taken with the help of a micropipette, passed through 0.45 μm filters and taken into HPLC vials. Samples containing chloramphenicol and tetracycline enriched in this way were analyzed by HPLC.

2.5. Milk samples and protein precipitation

Milk samples were prepared for the analysis by using a methodology explained by Agadellis et al. [8]. Five different milk samples were purchased from a local market in Sivas, Turkey and were stored daily at 4 °C. Non-pasteurized milk samples were used in the application of the method development. Separation of milk proteins before the MSPE procedure was carried out by using a known protocol with small modifications which was optimized in previous studies [28–30]. According to the used protocol for the protein precipitation: 5 mL of milk sample was homogenized and added 5 mL of ACN. After vortex mixing of the solution for 1 min, the samples were centrifuged for 10 min at 3500 rpm. Then, the supernatant in Falcon tubes was evaporated under nitrogen to a final volume of 5 mL and submitted to the developed MSPE procedure.

3. Results and discussions

3.1. Characterization of the C-nanofiber coated magnetic nanoparticles

XRD, Raman and FE-SEM analyzes were performed to verify the production of magnetic C-nanofibers. The formation of magnetic C-nanofibers with particle sizes below 200 nm was proven by FE-SEM images. When the images of the magnetic C-nanofiber material, which is the final product of the synthesis, are examined, homogeneous distribution of Fe_3O_4 structures on the fiber rods below 200 nm was seen (Fig. 1). X-ray diffraction (XRD) measurements were made to examine the crystal structure of magnetic C-nanofibers. As shown in Fig. 2, the observed diffraction peaks at 2θ: 25 and 43.0 for specific peaks of C-nanofiber. Again, the diffraction peaks at 2θ: of 30.2, 32, 35.8, 45.6, 57 and 63 are characteristic peaks of magnetic Fe_3O_4 particles [31,32]. The raman spectrum of magnetic carbon nanofibers and Fe_3O_4 nanoparticles were also obtained. As shown in Fig. 3, two intense characteristic peaks centered at 1342 cm^{-1} (D peak) and 1587 cm^{-1} (G peak) were observed for C-nanofibers. For Fe_3O_4, the characteristic peaks at 293, 409, 614 and 1315 cm^{-1} with their height were observed. The peaks obtained were confirmed to be compatible with the literature [33]. The I_D/I_G ratio representing the graphitization scope was calculated as 1.18 using peak heights based on Lorentzian function fit. Results from FE-SEM, XRD and Raman analyzes show that carbon nanofibers have been successfully magnetized by Fe_3O_4 nanoparticles formed homogeneously on *c*-nanofibers. In view of the Brunauer-Emmett Teller (BET) theory, C-nanofiber coated magnetic nanoparticles of the specific surface area, BJH Adsorption average pore width (4 V/A) and BJH adsorption cumulative volume of pores: was 25.7 $m^2\ g^{-1}$, 117.981 Å and 0.086403 $cm^3\ g^{-1}$, respectively.

3.2. Optimization experiments

3.2.1. Effect of pH

Sample solution pH is one of the most important parameters that affect the determination of the form in which the analyte is in the solution and therefore the retention of the analyte on the solid sorption phase. Therefore, it is one of the first parameters to be optimized. In order to find the most suitable pH, magnetic solid phase extraction method was applied to model solutions by adding phosphate buffer solutions changing pH from 2 to 11. According to the results shown in Fig. 4. As the solution pH moves from the acidic region to the basic region, the extraction efficiency increases, and the maximum extraction efficiency is obtained at pH 9.0 and the extraction efficiency decreases again at higher pH values. It was decided that pH 9.00 BR buffer will be used for further MSPE experiments.

3.2.2. Optimization of Adsorption and desorption time

Efficient contact of the adsorbent with sample solution containing the analyte(s) or the elution solutions are important criteria to reach high extraction efficiency for solid phase extraction. Manual shakers, vortex mixers and ultrasonic vibration apparatus used to increase the contact between the adsorbent and sample/elution solutions are effective laboratory equipment that can be found in almost any laboratory. For our experiments, the model solutions with the same conditions were prepared and other parameters were kept constant except the vortex

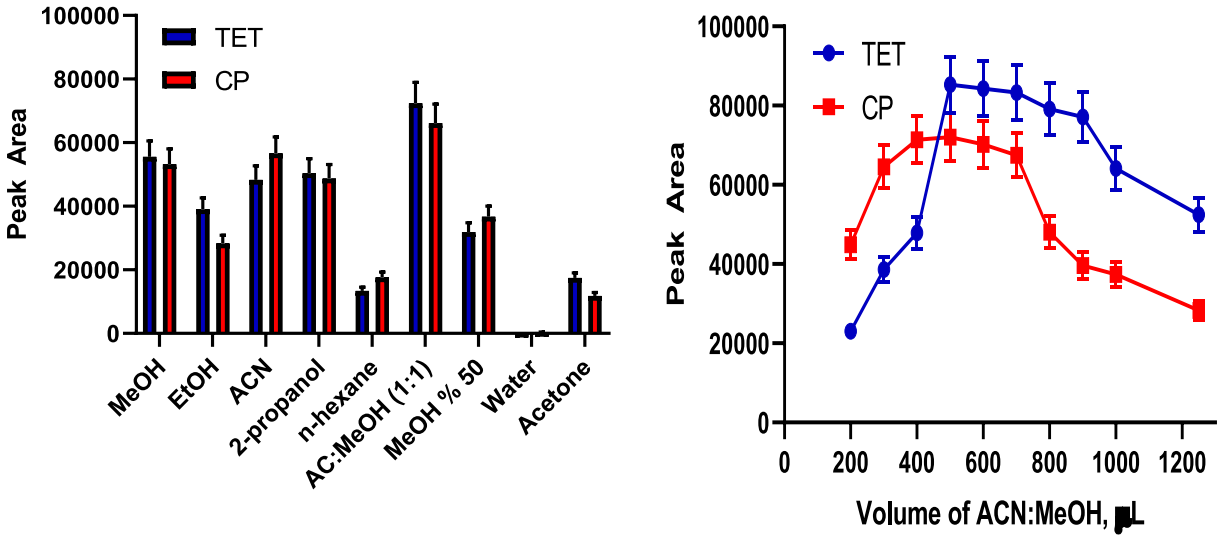

Fig. 6. a) The effect of elution solvents on desorption efficiency of TET and CP (N = 3) b) The effect of volume of elution solvent on desorption efficiency of TET and CP (N = 3).

Table 1
Analytical merits of the developed MSPE-HPLC method.

Parameter	Before MSPE HPLC-PDA		After MSPE HPLC-PDA	
	Chloramphenicol	Tetracycline	Chloramphenicol	Tetracycline
Linearity	2.0–20.0 μg mL^{-1}	2.0–20.0 μg mL^{-1}	10.0–600.0 ng mL^{-1}	10–600 ng mL^{-1}
LOD[a]	0.62 μg mL^{-1}	0.55 μg mL^{-1}	3.02 ng mL^{-1}	3.52 ng mL^{-1}
LOQ[b]	1.90 μg mL^{-1}	1.86 μg mL^{-1}	9.63 ng mL^{-1}	9.83 ng mL^{-1}
RSD (%)	4.0	3.5	3.2	3.8
Calibration Sensitivity	9.85	12.35	1221.42	1346.15
Determination Coefficient (R^2)	0.9972	0.9985	0.9954	0.9973
Preconcentration Factor (PF)	–	–	100	100
Enhancement Factor (EF)	–	–	124	109

[a] Limit of Detection.
[b] Limit of Quantification.

shaking time. It was studied for periods ranging from 0 to 90 min and the adsorption equilibrium was reached within 30 min (Fig. 5a). There appears to be no significant change in the extraction yield beyond 30 min. Therefore, in the following experiments, all samples were vortexed for 30 min. The same experiments were carried out to find optimum desorption time and results showed that the desorption equilibrium between adsorbent and elution solvent was reached within 20 s (Fig. 5b). There was no significant change in the elution efficiency above 20 s. Hence, analytes were desorbed with 20 s of vortex mixing for subsequent experiments.

3.2.3. *Eluent type and volume*

One of the most critical steps of the solid phase extraction method is that all or almost all of the analytes adsorbed on the adsorbent surface must be exhaustively desorbed. The choice of elution solvent to be used should be able to obtain the most ideal and distinctive peaks, provide a high desorption efficiency from the adsorbent, and be able to dissolve a high percentage of analyte. For this purpose, methanol, ethanol, isopropanol, acetonitrile, distilled water, acetone, hexane and methanol/acetone (ACN: MeOH) solution at 1:1 (*v/v*) ratio were evaluated as suitable solvents available in our laboratory. The results obtained were shown in Fig. 6a. The best desorption efficiency was achieved with the solvent ACN: MeOH (1:1). Therefore, this solution was selected as eluent for further studies. After the suitable elution solvent was determined, the volume of elution solvent was studied in order to obtain the highest extraction efficiency. The MSPE experiments were carried out on the model solutions under same test parameters by using ACN: MeOH (1:1) in volumes varying between 200 μL and 1250 μLas shown in Fig. 6b. The obtained results showed that the highest extraction efficiencies were obtained by eluting with 500 μL ACN: MeOH (1:1). Therefore, 500 μL ACN: MeOH (1:1) was used as the optimum eluent.

3.2.4. *The amount of adsorbent and reusability*

The developed material should possess strong affinity towards the target antibiotic molecules. Since, the current study focuses on trace determination of TET and CP molecules, the adsorption capacity is not the main interest of study. However, it is important to evaluate the minimum quantity of the adsorbent needed in the extraction process for facilitate the extraction process, efficiency of separation, and reusability of magnetic material. The effect of the adsorbent amount on the extraction efficiency was studied in the range of 10–100 mg. The MNPs was added to 50 mL of the sample solution. The obtained results showed that analytical signals were increased by the adsorbent amounts up to 50 mg, and then there was not a meaningful change and then remained constant. In addition, magnetic separation of MNPs after SPE by an external magnet was very easy for re-use.

The other important parameter is reusability of the developed materials. This directly affects both the analysis cost and repeatability. The reusability tests of the synthesized magnetic materials were performed to observe the stability of the adsorbent to be reused after extraction process. The evaluation was carried out by comparing peak areas of model samples including 100 ng mL^{-1} of both antibiotics. After 20 cycle use, the change of peak area for TET and CP molecules was lower than 10% of RSD. Magnetic material after every use was washed with 2 mL of

Table 2
Application of the proposed MSPE-HPLC procedure for milk samples (N = 3).

Samples[a]	Added ng mL^{-1}	Found[b] ng mL^{-1}		RSD %		Recovery %	
		CP	TET	CP	TET	CP	TET
Milk 1	0.0	Not	Not	–	–	–	–
	100.0	detected	detected	5.7	4.5	95.8	94.6
	300.0	95.8 ± 5.5	94.6 ± 4.3	5.0	4.2	97.1	98.1
		291.3 ± 14 .7	294.3 ± 12.4				
Milk 2	0.0	Not	Not	–	–	–	–
	100.0	detected	detected	4.4	4.7	98.7	96.4
	300.0	98.7 ± 4.4	96.4 ± 4.5	4.3	5.5	101.4	104.2
		304.3 ± 13.2	312.5 ± 17.2				
Milk 3	0.0	53.3 ± 3.4	Not	6.3	–	–	–
	100.0		detected	5.9	4.1	92.9	104.4
	300.0	142.5 ± 8.5	104.4 ± 4.3	5.1	5.3	97.0	101.4
		342.8 ± 17.7	304.3 ± 16.1				
	0.0	Not	Not	–	–	–	–
Milk 4	100.0	detected	detected	5.3	4.1	102.5	98.7
	300.0	102.5 ± 5.4	98.7 ± 4.0	5.2	5.9	104.1	95.7
		312.3 ± 16.2	285.9 ± 16.8				
	0.0	Not	75.8 ±	–	5.0	–	–
Milk 5	100.0	detected	3.8	6.7	3.6	95.9	105.4
	300.0	95.9 ± 6.4	185.4 ± 6.7	4.9	5.1	95.7	104.4
		287.3 ± 18.2	392.3 ± 20.3				

[a] The samples were prepared as explained in section.
[b] The average value of five replicates ± standard deviation.

ACN: MeOH (1:1) and 2 mL of ultrapure water. Thus, the synthesized magnetic material was proven as robust solid phase sorbent.

3.2.5. Analytical performance criteria of the method

After the determination of the most suitable experimental conditions, all analytical performance parameters of the developed MSPE-HPLC procedure including the enhancement factor (EF), pre-concentration factor (PF), relative standard deviation (RSD, %), limit of detection (LOD), limit of quantification (LOQ), linear range and correlation coefficient were determined, and reported in Table 1. The developed MSPE method was applied to standard solutions containing increasing concentrations of tetracycline (TET) and chloramphenicol (CP) antibiotics to determine the linear working range. The linear calibration curves were found for Tetracycline and Chloramphenicol in the range of 10.0–600.0 ng mL^{-1}. Linearity of method describes the direct proportionality between the concentration of TET and CP molecules in model solutions and peak area of antibiotics. 10 calibration standards in the range of 10.0–600.0 ng mL^{-1} were tested for 3 replicate analysis.

The proposed method was validated following the International Conference on Harmonization (ICH, 2005, ICH, 2019) and Food and Drug Administration (FDA, 2018) guidelines. The limit of detection (LOD) describes the lowest concentration of an analyte that can be reliably detected. The limit of quantitation (LOQ) refers to the lowest concentration of the analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. In accordance with ICH guidelines (Q2/R1 2005), the LOD and LOQ were derived from the slope m and its residual standard deviation of the response SD, derived from three calibration curves. Through the calibration curve parameters, the limits of detection (LOD) and limits of quantification (LOQ) were also calculated being LOD equal to calculated intercept of linear regression plus three times the Sy/x, and for LOQ, ten times this value [34,35]. Preconcentration factors (PF) were calculated by using the ratio of the initial solution volume (50 mL) to the last elution solvent volume. The enhancement factors (EF) were found from the ratio of the slope of calibration curve of the analytes after MSPE application to that of prior MSPE application. The relative standard deviations (RSD, %) were found by applying the MSPE method for seven repeating analysis, which includes 100 ng mL-1 of tetracycline and chloramphenicol.

3.2.6. Analysis of real samples

To investigate the applicability of the developed method on real samples and correctness of the developed method by means of recovery studies, milk samples were analyzed under the optimized conditions. According to Turkish Food Codex, maximum residue limits of CP and

Table 3
Comparison of the new method with other reported methods.

Pre-concentration Method	Determination Method	Target Molecule	LOD	Linear Range	Applications	Reference
Mixed-mode fabric phase sorptive extraction	HPLC-UV	Tetracycline	15 μg kg^{-1}	–	milk samples	[8]
ionic liquid-anionic surfactant based aqueous two-phase extraction	HPLC	Chloramphenicol Tetracycline	4.2 μg kg^{-1} 5.8 μg kg^{-1}	20.4–305.4 μg kg^{-1}	Honey	[37]
Matrix molecularly imprinted mesoporous sol–gel	LC-MS	Chloramphenicol	17 μg kg^{-1}	50–5000 μg kg^{-1}	milk samples	[38]
. Solid-phase extraction	HPLC-DAD	Chloramphenicol Tetracycline	21.4 ng. mL^{-1} 21.0 ng mL^{-1}	50–500 ng mL^{-1} 50–500 ng mL^{-1}	Bovine Milk	[39]
Direct Sample extraction	ELISA kit	Chloramphenicol Tetracycline	12.5 ng L^{-1} 1.500 ng L^{-1}	50–4.050 ng L^{-1}	ultra-heat-treatment milk	[40]
magnetic metal organic framework of type Fe_3O_4@ZIF-8	ultra-HPLC-MS	Tetracycline	0.125 ng mL^{-1}	0.5–50 ng L^{-1}	water samples	[10]
solid-phase extraction (with a C18 cartridge)	HPLC	Chloramphenicol Tetracycline	0.45 μg mL^{-1} 0.44 μg mL^{-1}	2–10 μg mL^{-1} 2–10 μg mL^{-1}	milk samples	[3]
Direct Sample extraction	LC-MS/MS	Tetracyclines (TCs)	17.2 μg kg^{-1}	–	Chicken meat.	[41]
molecularly Imprinted Polymer Mixed with Solid-Phase Extraction	HPLC	Chloramphenicol Tetracycline	20 μg kg^{-1} 10 μg kg^{-1}	10–1000 μg kg^{-1} 20–600 μg kg^{-1}	Raw Milk	[42]
Magnetic Solid Phase Extraction	HPLC-PDA	Chloramphenicol Tetracycline	3.43 ng mL^{-1} 3.55 ng mL^{-1}	10–600 ng mL^{-1} 10–600 ng mL^{-1}	Milk Samples	This Study

TET in the milk sample are zero and 100 μg kg^{-1} [36]. The CP and TET contents in the tested samples were shown in Table 2. In one of samples, TET was detected while CP was detected in another sample. The spiked recoveries of antibiotic molecules were in the ranges 94.6–105.4% for milk samples. These satisfactory results demonstrate that the proposed method of sample pretreatment and analysis is suitable for determining CP and TET antibiotics in milk samples.

3.3. Comparison of analytical merits

A comparison table is given in Table 3. The developed method has comparable merits with approaches MS based methods. As known, analysis cost is higher in MS based methods. The applicable linear range and facility of simultaneously analysis of two antibiotics with the developed method are highlights of this study. The most important advantage of the proposed method is to offer an easy applicable approach for two antibiotic molecules in milk samples by using a conventional HPLC and MSPE.

4. Conclusions

In the present work, C-nanofiber coated magnetic nanoparticles were explored as extraction sorbent for the MSPE toward TET and CP molecules at trace levels. The proposed approach exhibited good sensitivity, wide linearity, simple operation, and excellent recovery. This proposed approach was successfully employed for analyzing real milk samples. The nanocomposite exhibited promising performance in MSPE of antibiotics, and further exploration of the use of magnetic C-nanofiber material in the extraction strategy is in progress.

The results showed that this developed method is reliable and highly sensitive, and may meet the requirements for trace TET and CP molecules in milk samples. The residual concentration of antibiotics in the milk samples was relatively low, so it is necessary to enrich the analytes before analysis. The results indicate that the described method might to be a tool for the determination of antibiotics residues in the real samples, as well as providing useful information on the application of magnetic materials in the separation field.

Author contribution

Busra Vuran: Investigation, concept and design, experimental studies, Writing – original draft, reviewing and editing, Halil Ibrahim Ulusoy: Investigation, concept and design, experimental studies, Writing – original draft, reviewing and editing, Gokhan Sarp: Investigation, concept and design, experimental studies, Data curation, Writing – original draft, reviewing and editing, Erkan Yilmaz: Investigation, concept and design, Data curation, Conceptualization, Writing – original draft, reviewing and editing, Ummügülsüm Morgul: Investigation, concept and design, Data curation, Conceptualization, Writing – original draft, reviewing and editing, Abuzar Kabir: Investigation, Data curation, Writing – original draft, reviewing and editing, Angela Tartaglia: Investigation, concept and design, Data curation, Writing – original draft, reviewing and editing, Marcello Locatelli: Investigation, concept and design, experimental studies, Writing – original draft, reviewing and editing, Mustafa Soylak: Investigation, concept and design, Data curation, Conceptualization, Writing – original draft, reviewing and editing

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study has been partly supported by Cumhuriyet University Scientific Research Projects Commission as the research project with the ECZ-048 and ECZ-052 codes. In addition, this study was graduation project of Busra VURAN who was student in Faculty of Pharmacy.

References

[1] A. Pena, N. Pelantova, C.M. Lino, M.I.N. Silveira, P. Solich, Validation of an analytical methodology for determination of oxytetracycline and tetracycline residues in honey by HPLC with fluorescence detection, J. Agric. Food Chem. 53 (2005) 3784–3788, https://doi.org/10.1021/jf050065r.
[2] S. Babić, D. Ašperger, D. Mutavdžić, A.J.M. Horvat, M. Kaštelan-Macan, Solid phase extraction and HPLC determination of veterinary pharmaceuticals in wastewater, Talanta 70 (2006) 732–738, https://doi.org/10.1016/j.talanta.2006.07.003.
[3] S.M. Santos, M. Henriques, A.C. Duarte, V.I. Esteves, Development and application of a capillary electrophoresis based method for the simultaneous screening of six antibiotics in spiked milk samples, Talanta 71 (2007) 731–737, https://doi.org/10.1016/j.talanta.2006.05.049.
[4] S. Teixeira, C. Delerue-Matos, A. Alves, L. Santos, Fast screening procedure for antibiotics in wastewaters by direct HPLC-DAD analysis, J. Separ. Sci. 31 (2008) 2924–2931, https://doi.org/10.1002/jssc.200800229.
[5] A.L. Cinquina, F. Longo, G. Anastasi, L. Giannetti, R. Cozzani, Validation of a high-performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscle, J. Chromatogr. A 987 (2003) 227–233, https://doi.org/10.1016/S0021-9673(02)01446-2.
[6] T. Mk, Effect of biofield treatment on spectral properties of paracetamol and piroxicam, Chem. Sci. J. 6 (2015) 98, https://doi.org/10.4172/2150-3494.100098.
[7] C. Cháfer-Pericás, Á. Maquieira, R. Puchades, Fast screening methods to detect antibiotic residues in food samples, TrAC Trends Anal. Chem. (Reference Ed.) 29 (2010) 1038–1049, https://doi.org/10.1016/j.trac.2010.06.004.
[8] E. Agadellis, A. Tartaglia, M. Locatelli, A. Kabir, K.G. Furton, V. Samanidou, Mixed-mode fabric phase sorptive extraction of multiple tetracycline residues from milk samples prior to high performance liquid chromatography-ultraviolet analysis, Microchem. J. 159 (2020) 105437, https://doi.org/10.1016/j.microc.2020.105437.
[9] J.W. Fritz, Y. Zuo, Simultaneous determination of tetracycline, oxytetracycline, and 4-epitetracycline in milk by high-performance liquid chromatography, Food Chem. 105 (2007) 1297–1301, https://doi.org/10.1016/j.foodchem.2007.03.047.
[10] H. Liu, L. Chen, J. Ding, A core-shell magnetic metal organic framework of type Fe3O4@ZIF-8 for the extraction of tetracycline antibiotics from water samples followed by ultra-HPLC-MS analysis, Microchim. Acta 184 (2017) 4091–4098, https://doi.org/10.1007/s00604-017-2442-6.
[11] S. Valenti, A. Diaz, M. Romanini, L.J. del Valle, J. Puiggalí, J.L. Tamarit, R. Macovez, Amorphous binary dispersions of chloramphenicol in enantiomeric pure and racemic poly-lactic acid: morphology, molecular relaxations, and controlled drug release, Int. J. Pharm. 568 (2019) 118565, https://doi.org/10.1016/j.ijpharm.2019.118565.
[12] L.M. Namegabe, S.O. Sarr, Y.M. Diop, Development and validation of a spectrofluorimetric method for the assay of tetracycline in capsules, Am. J. Anal. Chem. 9 (2018) 162–170, https://doi.org/10.4236/ajac.2018.93014.
[13] B.K. Kolb, L. Riesterer, L. Bier, A.M. Widenhorn, Proof of bacteria and the activity of chemical and natural antibiotics by headspace gas chromatography, J. Anal. Sci. Technol. 10 (2019) 1–9, https://doi.org/10.1186/s40543-019-0167-3.
[14] X.D. Zhu, Y.J. Wang, R.J. Sun, D.M. Zhou, Photocatalytic degradation of tetracycline in aqueous solution by nanosized TiO2, Chemosphere 92 (2013) 925–932, https://doi.org/10.1016/j.chemosphere.2013.02.066.
[15] V. Andreu, P. Vazquez-Roig, C. Blasco, Y. Picó, Determination of tetracycline residues in soil by pressurized liquid extraction and liquid chromatography tandem mass spectrometry, Anal. Bioanal. Chem. 394 (2009) 1329–1339, https://doi.org/10.1007/s00216-009-2635-x.
[16] H.I. Ulusoy, Applications of Magnetic Nanoparticles for the Selective Extraction of Trace Species from a Complex Matrix, first ed., Nova Scientific Publishers, New York, 2017.
[17] M. Locatelli, K.G. Furton, A. Tartaglia, E. Sperandio, H.I. Ulusoy, A. Kabir, An FPSE-HPLC-PDA method for rapid determination of solar UV filters in human whole blood, plasma and urine, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1118 (2019) 40–50, https://doi.org/10.1016/j.jchromb.2019.04.028.
[18] M. Soylak, L. Elci, Solid phase extraction of trace metal ions in drinking water samples from Kayseri-Turkey, J. Trace Microprobe Tech. 18 (2000) 397–403.
[19] M.C. Vargas Mamani, F.G. Reyes Reyes, S. Rath, Multiresidue determination of tetracyclines, sulphonamides and chloramphenicol in bovine milk using HPLC-DAD, Food Chem. 117 (2009) 545–552, https://doi.org/10.1016/j.foodchem.2009.04.032.
[20] S. Ulusoy, S. Erdogan, M.G. Karaaslan, B. Ateş, H.İ. Ulusoy, S. Erdemoglu, Optimization of extraction parameters for folic acid and antioxidant compounds from an edible plant (polygonum cognatum meissn) using pressurized liquid extraction (PLE) system, Cumhur. Sci. J. 39 (2018) 1069–1080, https://doi.org/10.17776/csj.460289.
[21] H.İ. Ulusoy, Applications of magnetic nanoparticles for the selective extraction of trace species from a complex matrix, in: M. Locatelli, C. Celia (Eds.), Anal. Chem. Dev. Appl. Challenges Food Anal., 2017, pp. 55–75.

[22] S. Mahpishanian, H. Sereshti, Three-dimensional graphene aerogel-supported iron oxide nanoparticles as an efficient adsorbent for magnetic solid phase extraction of organophosphorus pesticide residues in fruit juices followed by gas chromatographic determination, J. Chromatogr. A 1443 (2016) 43–53, https://doi.org/10.1016/j.chroma.2016.03.046.
[23] X. Liu, T. Feng, C. Wang, L. Hao, C. Wang, Q. Wu, Z. Wang, A metal-organic framework-derived nanoporous carbon/iron composite for enrichment of endocrine disrupting compounds from fruit juices and milk samples, Anal. Methods. 8 (2016) 3528–3535, https://doi.org/10.1039/c6ay00191b.
[24] A. Kabir, M. Locatelli, H. Ulusoy, Recent trends in microextraction techniques employed in analytical and bioanalytical sample preparation, Separations 4 (2017) 36, https://doi.org/10.3390/separations4040036.
[25] H. Eskandari, A. Naderi-Darehshori, Preparation of magnetite/poly(styrene-divinylbenzene) nanoparticles for selective enrichment-determination of fenitrothion in environmental and biological samples, Anal. Chim. Acta 743 (2012) 137–144, https://doi.org/10.1016/j.aca.2012.07.012.
[26] J. Wang, L. Hao, C. Wang, Q. Wu, Z. Wang, Nanoporous carbon as the solid-phase extraction adsorbent for the extraction of endocrine disrupting chemicals from juice samples, Food Anal. Methods 10 (2017) 2710–2717, https://doi.org/10.1007/s12161-017-0828-z.
[27] Y. Song, R. Ma, C. Jiao, L. Hao, C. Wang, Q. Wu, Z. Wang, Magnetic mesoporous polymelamine-formaldehyde resin as an adsorbent for endocrine disrupting chemicals, Microchim. Acta 185 (2018) 19, https://doi.org/10.1007/s00604-017-2593-5.
[28] Y. Wang, T. Xie, J. Yang, M. Lei, J. Fan, Z. Meng, M. Xue, L. Qiu, F. Qi, Z. Wang, Fast screening of antibiotics in milk using a molecularly imprinted two-dimensional photonic crystal hydrogel sensor, Anal. Chim. Acta 1070 (2019) 97–103, https://doi.org/10.1016/j.aca.2019.04.031.
[29] V. Samanidou, K. Michaelidou, A. Kabir, K.G. Furton, Fabric phase sorptive extraction of selected penicillin antibiotic residues from intact milk followed by high performance liquid chromatography with diode array detection, Food Chem. 224 (2017) 131–138, https://doi.org/10.1016/j.foodchem.2016.12.024.
[30] K. Saridal, H.İ. Ulusoy, A simple methodology based on cloud point extraction prior to HPLC-PDA analysis for tetracycline residues in food samples, Microchem. J. 150 (2019) 104170, https://doi.org/10.1016/j.microc.2019.104170.
[31] Z. Zhang, X. Deng, J. Sunarso, R. Cai, S. Chu, J. Miao, W. Zhou, Z. Shao, Two-step fabrication of Li4Ti5O12-coated carbon nanofibers as a flexible film electrode for high-power lithium-ion batteries, ChemElectroChem 4 (2017) 2286–2292.
[32] E. Yilmaz, G. Sarp, Graphene-like MoS 2-modified magnetic C-dot nanoflowers: an efficient magnetic solid-phase extraction adsorbent for monitoring of trace amounts of ibuprofen, Anal. Methods. 12 (2020) 1570–1578.
[33] E. Yilmaz, S. Salem, G. Sarp, S. Aydin, K. Sahin, I. Korkmaz, D. Yuvali, TiO2 nanoparticles and C-Nanofibers modified magnetic Fe3O4 nanospheres (TiO2@ Fe3O4@ C–NF): a multifunctional hybrid material for magnetic solid-phase extraction of ibuprofen and photocatalytic degradation of drug molecules and azo dye, Talanta 213 (2020) 120813.
[34] I.M.P.L.V.O. Ferreira O. Viegas, C. Esteves, J. Rocha, A. Melo, Simultaneous determination of melatonin and trans-resveratrol in wine by dispersive liquid-liquid microextraction followed by HPLC-FLD, Food Chem. 339 (2020) 128091, https://doi.org/10.1016/j.foodchem.2020.128091.
[35] ICH, The international conference on harmonization. Harmonized tripartite guideline. Validation of analytical procedures: text and methodology Q2(R1)., ICH harmon. Tripart. Guidel. Valid, Anal. Proced. TEXT Methodol. Q2 (R1) (2005), 1.20: 05.
[36] Turkish Food Codex, Ankara, Gida Maddelerinde Belirli Bulasanlarin Maksimum Seviyelerinin Belirlenmesi Hakkinda Teblig, vol. 23, 2002.
[37] X. Yang, S. Zhang, W. Yu, Z. Liu, L. Lei, N. Li, H. Zhang, Y. Yu, Ionic liquid-anionic surfactant based aqueous two-phase extraction for determination of antibiotics in honey by high-performance liquid chromatography, Talanta 89 (2014) 201–208, https://doi.org/10.1016/j.talanta.2014.02.039.
[38] V. Samanidou, M. Kehagia, A. Kabir, K.G. Furton, Matrix molecularly imprinted mesoporous sol-gel sorbent for efficient solid-phase extraction of chloramphenicol from milk, Anal. Chim. Acta 914 (2016) 62–74, https://doi.org/10.1016/j.aca.2016.02.003.
[39] P. Moudgil, J.S. Bedi, R.S. Aulakh, J.P.S. Gill, A. Kumar, Validation of HPLC multi-residue method for determination of fluoroquinolones, tetracycline, sulphonamides and chloramphenicol residues in bovine milk, Food Anal. Methods 11 (2019) 1944–1950, https://doi.org/10.1007/s12161-018-1365-0.
[40] N. Unusan, Occurrence of chloramphenicol, streptomycin and tetracycline residues in ultra-heat-treatment milk marketed in Turkey, Int. J. Food Sci. Nutr. 60 (2009) 359–364, https://doi.org/10.1080/09637480701664555.
[41] F. Cetinkaya, A. Yibar, G.E. Soyutemiz, B. Okutan, A. Ozcan, M.Y. Karaca, Determination of tetracycline residues in chicken meat by liquid chromatography-tandem mass spectrometry, Food Addit. Contam. Part B Surveill. 5 (2012) 45–49, https://doi.org/10.1080/19393210.2012.655782.
[42] Y. Xie, Q. Hu, M. Zhao, Y. Cheng, Y. Guo, H. Qian, W. Yao, Simultaneous determination of erythromycin, tetracycline, and chloramphenicol residue in raw milk by molecularly imprinted polymer mixed with solid-phase extraction, Food Anal. Methods 11 (2018) 374–381, https://doi.org/10.1007/s12161-017-1008-x.