Received: 31 August 2018 | Revised: 31 October 2018 | Accepted: 4 November 2018

DOI: 10.1002/jssc.201800901

RESEARCH ARTICLE

JOURNAL OF SEPARATION SCIENCE

Simultaneous determination of selected estrogenic endocrine disrupting chemicals and bisphenol A residues in whole milk using fabric phase sorptive extraction coupled to HPLC-UV detection and LC-MS/MS

Rodolfo Mesa[1] | Abuzar Kabir[1]* | Victoria Samanidou[2] | Kenneth G. Furton[1]

[1]Department of Chemistry and Biochemistry, International Forensic Research Institute, Florida International University, Miami, USA

[2]Department of Chemistry, Laboratory of Analytical Chemistry, Aristotle University of Thessaloniki, Greece

Correspondence
Dr. Victoria Samanidou, Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Greece.
Email: samanidu@chem.auth.gr

*Additional corresponding author:
Dr. Abuzar Kabir
Email: akabir@fiu.edu

A simple and sensitive analytical methodology is developed for rapid screening and quantification of selected estrogenic endocrine disrupting chemicals and bisphenol A from intact milk using fabric phase sorptive extraction in combination with high-performance liquid chromatography coupled to ultraviolet detection/tandem mass spectrometry. The new approach eliminates protein precipitation and defatting step from the sample preparation workflow. In addition, the error prone and time-consuming solvent evaporation and sample reconstitution step used as the sample post-treatment has been eliminated. Parameters with most significant impact on the extraction efficiency of fabric phase sorptive extraction including sorbent chemistry, sample volume, extraction time have been thoroughly studied and optimized. Separation of the selected estrogenic endocrine disrupting chemicals including α-estradiol, hexestrol, estrone, 17α-ethinyl estradiol, diethylstilboestrol, and bisphenol A were achieved using a Zorbax Extend-C18 high-performance liquid chromatography column (15 cm × 4.6 mm, 5 μm particle size). The limit of detection values obtained in fabric phase sorptive extraction with high-performance liquid chromatography with ultraviolet detection ranged from 25.0 to 50.0 ng/mL. The method repeatability values were 3.6–13.9 (relative standard deviation, %) and intermediate precision values were 4.6–12.7 (relative standard deviation, %). The fabric phase sorptive extraction method was also coupled to liquid chromatography with tandem mass spectrometry for identifying each endocrine disrupting chemical at 10 ng/mL.

KEYWORDS
fabric phase sorptive extraction, green analytical chemistry, sample preparation, sol–gel processing, endocrine disrupting chemicals

1 | INTRODUCTION

Milk represents one of the most universally consumed foods in the world. As a relatively inexpensive but reliable source of many vital nutrients including saturated fat, protein, minerals, vitamins and others, the importance of milk in human life cannot be overemphasized. However, various synthetic steroid hormones are often illicitly administered to milking animals as growth promoters in order to gain quick physical growth and enhanced milk production. In addition, some natural steroid hormones from the milking animals may spontaneously enter the milk. These natural and synthetic steroid hormones belong to a special class of compounds known as endocrine disrupting chemicals (EDCs) [1]. EDCs are

Article Related Abbreviations: 17α-EE2, 17α-ethinyl estradiol; DES, diethylstilboestrol; E1, estrone; EDC, endocrine disrupting chemical; FPSE, fabric phase sorptive extraction; HEX, hexestrol; MTMS, methyltrimethoxysilane; PDMS, poly(dimethyl siloxane); PTHF, poly(tetrahydrofuran); SBSE, stir-bar sorptive extraction

exogenous substances capable of modifying the function of the endocrine system, and consequently cause many detrimental effects to the health of humans. Adverse effects directly attributed to EDCs include altering reproductive function in males and females, increased cases of breast cancer, abnormality in growth patterns, delays in neuron development, and changes in immune function. Some of these compounds can be carcinogenic even at a very low concentration [2]. Considering the dire consequences attributed to the exposure of these EDCs, regulatory agencies such as the European Union imposed maximum residue limits for veterinary substances administered to food-producing animals as part of Council Regulation 37/2010/EC and have also set forth guidelines for analytical method performance in the determination of organic residues in animal products [3,4]. These guidelines can be found in the 657/2002/EC European Union Commission Decision [1].

Another EDC of high significance is bisphenol A (BPA), which possesses activity similar to estrogenic steroids. BPA is an industrial chemical, widely used in the production of polycarbonate plastics, epoxy resins and as a stabilizer in polyvinyl chloride [5]. Polycarbonate plastics are commonly used in food and drink packaging; resins are used as the lining material to coat metal products such as food cans, bottle tops, and milk containers. The migration of BPA from epoxy lined can surfaces, polycarbonate plastics, and PVC products into food has been already reported [6].

Despite the strong push by the regulatory agencies making it a priority to ensure the safety of milk and dairy products for consumption, commercially available methods are inadequate to reliably and accurately assess the safety of these foods. This is partly due to the excessive complexity of milk as a sample matrix containing hundreds of interfering compounds, and partly due to the ultra-trace levels of concentration of the EDCs in milk. As such, a robust sample preparation strategy is inevitable that may efficiently minimize the matrix interference, preconcentrate the EDCs, and exchange solvent that is compatible with the chromatographic system. Major sample preparation techniques currently being used to monitor the presence of these EDCs in milk include: polymer monolith microextraction [7], stir-bar sorptive extraction (SBSE) [8], molecular imprinted polymer microspheres [9,10], restricted access molecularly imprinted polymer [11], LLE [12–14], LLE followed by C18 SPE [15], matrix solid-phase dispersion [16], miniaturized graphene-based pipette tip extraction [17], multiple monolithic fiber SPME [18], hollow-fiber-LPME [19], and SPE [14,20,21]. Although SPE is considered as the gold standard sample preparation technique for milk analysis [22], it requires defatting and protein precipitation of milk prior to extraction to mitigate the risk of SPE cartridge clogging. However, defatting and protein precipitation processes are not only time consuming and cumbersome but also lead to substantial analyte loss and therefore should be avoided. Considering the extraordinarily high consumption of milk worldwide, sample preparation techniques directed towards milk quality assurance and safety monitoring should be fast, simple, and inexpensive without requiring any sample pre-treatment steps such as filtration, centrifugation, defatting, and protein precipitation or post-treatment steps such as solvent evaporation and sample reconstitution in a suitable solvent.

Fabric phase sorptive extraction (FPSE) [23,24], a new generation sample preparation technique, has eloquently addressed the majority of the shortcomings pertaining to commercially available sample preparation techniques such as SPE and SPME. FPSE has uniquely combined SPE and SPME into a single sample preparation technique. It utilizes a piece of natural or synthetic fabric (cellulose/polyester/fiber glass) as the substrate to form a thin film of sol-gel hybrid inorganic-organic polymeric sorbent on its surface. During the sol-gel coating, the thin film chemically bonds to the substrate. The sol-gel coated FPSE media is highly porous, easily permeable and can be introduced directly into the sampling container for analyte extraction. A magnetic stirrer can be used to diffuse the analytes faster from the bulk solution to the FPSE media, resulting in faster mass transfer and shorter extraction equilibrium time. Due to the sponge-like porous architecture of the coating and permeable fabric substrate, aqueous sample matrices easily permeate through the FPSE media during extraction and accelerate analyte-extraction sorbent interaction for rapid extraction. At the end of the extraction, the FPSE media is exposed to a small volume of organic solvent (500 μL) for eluting the extracted analytes into it. If any fat or protein molecules are physically adhered to the FPSE media, they precipitate out during solvent back-extraction. Finally, the solution can be centrifuged to eliminate any particulate present in the solution and is subsequently injected into the chromatographic system. Applications of FPSE in milk analysis to monitor residual sulfonamide antibiotic drug residues [25], bisphenol A, and residual dental restorative material [26], amphenicols residues [27], penicillin antibiotic residues [28], and in biological fluids such as whole blood, plasma, and urine [29–31] have already demonstrated the advantages of FPSE over conventional sample preparation techniques.

As it pertains to instrumental analysis, the majority of methods published in literature use some variation of LC-MS [6,7,32,33], or GC-MS [34] for analysis of EDCs in milk. These instrument, however, are incapable of analysing milk samples without proper sample preparation.

The aim of the current study is to develop integrated FPSE-HPLC-UV and FPSE-LC-MS/MS methods capable of monitoring the presence of EDCs directly from intact milk without employing any sample pre-treatment exercises such as defatting and protein precipitation as well as post-treatment steps such as solvent evaporation and sample reconstitution

and to offer a fast, simple, and inexpensive alternative to the methodologies currently used in both dairy quality assurance and regulatory monitoring programs. α-Estradiol (α-E2) and estrone (E1) were selected as the representative natural estrogenic EDCs; 17α-ethinyl estradiol (17α-EE2), diethylstilboestrol (DES), bisphenol A (BPA) and hexestrol (HEX) were selected as the representative synthetic estrogenic EDCs as the model EDCs. Chemical structures, origins, and octanol-water coefficient (logKow) values of the selected EDCs are presented in Supporting Information Table S1. To the best of the authors' knowledge, this is the first report dedicated to monitoring EDCs directly from intact milk without defatting and protein precipitation.

2 | MATERIALS AND METHODS

2.1 | Instrumentation

The HPLC method development and validation was primarily performed on an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a degasser (G1322A), a quaternary pump (G1311A), an automatic liquid sampler (G1313A), a thermostated column compartment (G1316A), and a variable wavelength detector (G1314A). The HPLC separation column used was a Zorbax Extend-C18 (150 × 4.6 mm, 5 μm particle size), purchased from Agilent Technologies (Santa Clara, CA, USA). An Eppendorf Centrifuge 5415 R (Eppendorf North America, Hauppauge, NY, USA) was used to remove micro particles from the sol solutions prior to sol-gel coating on FPSE media and from the analyte solutions prior to injecting into the HPLC system. On-line data collection and processing of HPLC data was done using ChemStation software (Revision A.08.03) for Windows (Agilent Technologies, Santa Clara, CA, USA). A Philips XL30 Scanning Electron Microscope equipped with an EDAX detector was used to obtain SEM images. A Barnstead NANOPure Diamond (Model D11911) deionized water system (Barnstead, Dubuque, IA, USA) was employed to obtain ion-free water (18.0 MΩ). The HPLC-MS/MS analysis was conducted on a Thermo Finnigan Surveyor coupled to an Applied Biosystems QTRAP 5500 (Thermo Finnigan, San Jose, CA, USA) using the same HPLC mentioned before.

2.2 | Materials and reagents

All chemicals, reagents, solvents, organic polymers, and sol-gel precursors used in the current study were of the highest quality available in the market. Methyltrimethoxysilane (MTMS), trifluoroacetic acid (TFA), acetone, polytetrahydrofuran (PTHF), dichloromethane, estradiol, HEX, E1, 17α-EE2, DES, and bisphenol A (BPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide and hydrochloric acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). The HPLC-grade solvents, water and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA, USA). Muslin cotton fabric (100% cellulose) was purchased from Jo-Ann Fabric (Miami, FL, USA).

2.3 | Preparation of sol-gel sorbent coated FPSE media

Muslin cotton (100% cellulose) fabric was used as the substrate for sol-gel sorbent coating. Prior to the sol-gel coating, commercial cotton fabric was treated chemically in order to clean the substrate from residual surface finishing chemicals as well as to maximize surface hydroxyl functional groups. A detailed treatment process of the fabric substrate can be found elsewhere [23,35].

A medium polarity organic polymer, poly(tetrahydrofuran) (PTHF) and a low polarity organic polymer, poly(dimethyl siloxane (PDMS) were used to create sol-gel PTHF and sol-gel PDMS coated FPSE media, respectively. The formulation of the sol solutions for sol-gel PTHF and sol-gel PDMS has been described elsewhere [35]. Briefly, the sol solution was prepared by sequential addition of the ingredients: 12.5 g PTHF polymer; 12.5 mL acetone; 12.5 mL methylene chloride; 12.5 mL methyl trimethoxysilane; and 5 mL trifluoroacetic acid (impregnated with 5% water). The sol solution was vortexed for 2 min. Immediately after adding the sol-gel catalyst, trifluoroacetic acid, the sol solution was vortexed for 3 min, centrifuged for 5 min, and solicited for 5 min. Subsequently, the particle and gas free supernatant sol solution was transferred into a 2 oz amber glass reaction bottle. A 20 × 10 cm piece of clean and chemically treated cotton fabric substrate was immersed into the sol solution to initiate the surface sol-gel sorbent coating. The fabric was kept inside the sol solution for 4 h.

At the end of the sol-gel coating, the coated fabric was removed from the reaction bottle and was placed in a desiccator overnight. Subsequently, the sol-gel PTHF and sol-gel PDMS coated FPSE media were rinsed with an acetone: methylene chloride mixture (50:50; v/v) under continuous sonication for 30 min. The sol-gel PTHF and sol-gel PDMS coated FPSE media were then dried in ambient air and cut into 2.5 × 2.0 cm pieces. Finally, the small pieces of FPSE media were stored in an air-tight closed container.

2.4 | Sample collection and storage conditions

Milk samples used in the current study include whole milk (3.25% fat), reduced fat milk (2.0% fat), low fat milk (1% milk). and skim milk (0–0.5% fat). These milks were purchased from a local grocery store in Miami, FL. The samples were kept refrigerated at 4°C and never used more than

72 hours after being purchased or beyond the expiration dated printed on their labels.

2.5 | Preparation of standards and spiked milk samples

Primary standard solutions were prepared by dissolving each EDC in acetonitrile at a concentration of 10000 µg/mL, except for E1, which was prepared at a concentration of 2000 µg/mL. Intermediate solutions of all six analytes were used for further dilution or to spike the samples for extraction. Dilutions of standard solutions for LC method development or spiking of samples were always kept at a minimum to reduce potential error. Standards and intermediates were always kept refrigerated. Whole milk with its highest fat content (3.25% fat) poses a formidable challenge to the separation scientists as a sample matrix especially when the presence of pollutants at their trace or ultra-trace level concentration is needed to monitor. As such, whole milk was used as the sample matrix to spike with the target analytes during the method development and validation exercises. The analytical figures of merit are obtained using whole milk as the sample matrix. It is worthy to mention that, unlike some of the reported methods, whole milk samples were not diluted and used as received.

2.6 | Instrumental analysis by HPLC-UV detection and LC-MS/MS

The first step in the method development process was to develop a robust HPLC method. This was done by injecting standard solutions of the EDCs individually to confirm the ability of the instrument to detect each analyte and the elution order through the selected Zorbax Extend-C18 column. Once the elution order was determined, satisfactory resolution of the peaks was achieved with an isocratic method (55% water, 45% acetonitrile; flow rate 1 mL/min; column temperature 30°C; VWD set at 200 nm). A representative HPLC-UV chromatogram has been presented in Supporting Information Figure S2.

The chromatography portion of the HPLC-UV method was also used for analysis on an LC-MS/MS. Supporting Information Figure S3 represents the extracted ion chromatograms of the six EDCs. The instrument was used in Multiple-Reaction-Monitoring mode. Two transitions were monitored for each analyte.

3 | RESULTS AND DISCUSSION

3.1 | Rational selection of appropriate sorbent chemistry for FPSE

Sol-gel coating technology [36] offers a facile pathway to create surface coating of extraction sorbents possessing unique selectivity by a judicious selection of the sol solution ingredients primarily comprised of an organic polymer, a sol-gel precursor, a suitable solvent system and a sol-gel catalyst. Unlike pristine organic polymers commonly used in conventional microextraction techniques such as SPME and stir bar sorptive extraction (SBSE), sol-gel coating technology used in FPSE provides unique flexibility to design and fine tune the ultimate selectivity of the extraction medium by selecting the most appropriate organic polymer, sol-gel precursor, and fabric substrate. As such, FPSE simultaneously exploits the material properties of the organic polymer, inorganic sol-gel precursor, and the fabric substrate chemistry that collectively contribute to determine the overall selectivity and extraction sensitivity of the FPSE media.

Due to the medium and low polarity of the selected EDCs as shown in Supporting Information Table S1, a medium polar organic polymer, PTHF; an organically modified sol-gel precursor, methyl trimethoxysilane; two organic solvents: acetone and methylene chloride; and a sol-gel catalyst, trifluoroacetic acid (impregnated with 5% water, v/v) were selected to prepare the sol solution. This process was replicated with a nonpolar polymer, polydimethylsiloxane (PDMS), instead of PTHF to determine the better sorbent for extraction of selected EDCs.

In addition to the tunable selectivity, sol-gel coating technology offers a number of advantages: (a) cost-effectiveness; (b) molecular level uniformity in the coating; (c) chemical bonding between the coating and the fabric surface via exposed hydroxyl groups; (d) superior solvent, chemical, and thermal stability due to the inorganic component in the network; (e) resistance to physical stress such as scraping and bending; and (f) high primary contact surface area because of the flat geometry of the FPSE medium [23,36]. The chemical bonding between the sorbent and the substrate in FPSE medium plays an important role in its robustness and reproducible performance even after many repeated applications. Supporting Information Figure S1 represents a schematic diagram of sol-gel PTHF coated FPSE media.

3.2 | Optimization of FPSE parameters

The extraction efficiency of FPSE primarily depends on a number of important factors including the coating chemistry, sample volume, extraction time, stirring speed, desorption solvent, and desorption time. These factors were carefully optimized during the method development in order to maximize the extraction efficiency. Since whole milk represents the most complex sample matrix among all types of milk samples used in the current study, it was used throughout the FPSE method optimization study. Pre-optimization parameter values were: whole milk, 10 mL; extraction time, 60 min; stirring speed, 1150 rpm; elution solvent, acetonitrile; elution solvent volume, 500 µL; and elution time, 10 min.

Optimization experiments were carried out in triplicate with one factor changed at a time.

3.2.1 | Selection of the FPSE sorbent coating

Between the two sol-gel sorbent coatings tested to determine the better sorbent coating for the selected EDCs, sol-gel PTHF and sol-gel PDMS, sol-gel PTHF was distinctly superior in extraction efficiencies for all compounds (Figure 1A). The extraction efficiency values strongly correlated with the logKow value of the compounds, with BPA (logKow 3.32) showing the most difference between the two coatings and DES (logKow 5.07) showing the least difference. Although a nonpolar analyte such as DES should have been better extracted by the less polar sorbent (sol-gel PDMS), the results show otherwise. The origin of the disparity may have originated from the hydrophobic nature of the PDMS coating; fat molecules in milk felt affinity towards the sol-gel PDMS coating and covered a significant portion of the FPSE media surface, resulting in a lower primary contact surface area for analyte-sorbent interactions and subsequent extraction. On the other hand, it is unlikely that medium polar sol-gel PTHF retained many fat molecules due to its hydrophilicity. In addition, sol-gel PTHF coating is capable of interacting with analytes via hydrogen bonding. Regardless of the polarity of the EDCs investigated in the current study, all possess hydrogen bond donors and/or acceptors. As a result, both medium polar and nonpolar analytes were extracted better in sol-gel PTHF coated FPSE media. Based on this observation, sol-gel PTHF coated FPSE media was considered as the better sorbent for all subsequent experiments.

3.2.2 | Optimization of sample volume

Although FPSE is considered an equilibrium based sorptive microextraction technique like SPME, substantially high sorbent loading in FPSE media (~20 mg on a 5 cm^2 unit of sol-gel PTHF coated media), compared to SPME (~0.5 mg) and flow-through extraction mechanism like solid phase extraction, FPSE provides almost exhaustive extraction. As such, sample volume in FPSE plays an important role in determining the overall sensitivity of the method and often the optimum sample volume is determined to ensure maximum sensitivity of the method. The amount of analytes extracted in FPSE is expected to increase with the increase of the sample volume up to a certain value, after which no further increase is expected. In order to establish the optimum volume of milk, FPSE was used in 5, 10, and 15 mL of spiked whole milk possessing individual analyte concentration at 50 ng/mL for 1 h extraction at 1150 rpm stirring speed followed by eluting the analytes in 500 µL acetonitrile for 10 min. The data presented in Figure 1B reveals that all analytes except E1 showed higher extraction sensitivity at 5 mL extraction volume compared to 10 and 15 mL sample volume. The lower extraction efficiency at relatively higher sample volume may be attributed to the high content of fat molecules in milk, which not only reduced the accessible surface of the FPSE media for sorbent-analyte interaction, but also were affinitive towards the nonpolar analytes via hydrophobic-lipophilic interactions. The loss in extraction efficiency of different EDCs at higher sample volume were strongly correlated to their logKow values, with BPA (logKow 3.32) being the least affected and DES (logKow 5.07) being the most affected. As such, 5 mL milk was taken as the optimum volume for subsequent experiments.

3.2.3 | Optimization of extraction time

Extraction time in FPSE is among a few parameters that significantly influence the extraction efficiency and therefore it must be experimentally determined. Optimum extraction time primarily depends on the number of factors including molar mass of the analyte, polarity of the analyte, and the diffusion of analyte into the sample matrix during extraction, viscosity of the sample matrix, and other factors. To this end, a series of incrementally higher extraction times including 10, 20, 30, 40, 50, and 60 min were investigated. The results are shown in Figure 1C. As the data revealed, most of the EDCs reached 90% of their maximum extraction within 30 min. BPA needed 50 min to attain 90% of its maximum extraction. As such, 50 min was adopted as the optimum extraction time for rest of the experiments. Although 50 min extraction time seems to be long for FPSE, the relatively high viscosity of whole milk inhibits the fast diffusion of the analytes through the sample matrix. In addition, the presence of high volume of fat molecules in the whole milk occupies some of the interaction sites on the FPSE media for rapid analyte-sorbent interaction, resulting in a slow mass transfer kinetic and prolonged extraction equilibrium time.

3.2.4 | Optimization of stirring speed

Similar to other sorbent-based sorptive microextraction techniques, extraction equilibrium time in FPSE can be substantially reduced if the samples are diffused by external stimuli such as sonication, magnetic stirring and orbital shaking. These external stimuli rapidly diffuse the analyte into the sample matrix and the boundary layer on the FPSE media so that the boundary layer is never depleted of the analyte. Among all the external stimuli commonly used, magnetic stirring using a cylindrical bar magnet is the most common. The impact of the stirring speed on extraction efficiency was investigated at three different speeds: 600, 800, and 1150 rpm. As the results presented in Figure 1D show, both 800 and 1150 rpm provided similar extraction sensitivity; however, the reproducibility was better for 800 rpm stirring speed. Due to the relatively low volume of milk (5 mL), a higher stirring speed made the solution very turbulent and that may have contributed to the poor reproducibility between replicate extrac-

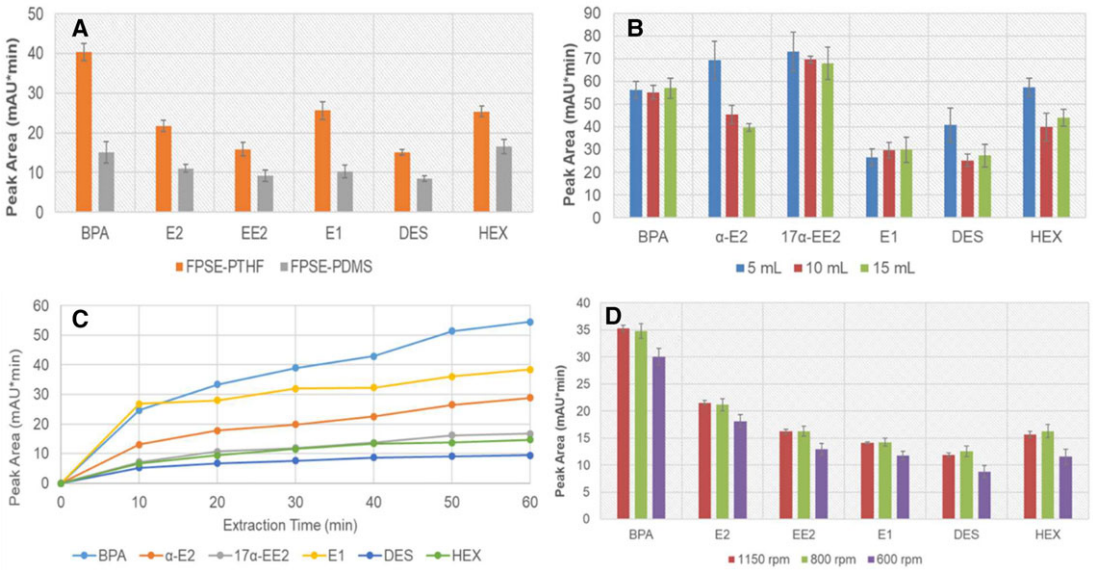

FIGURE 1 Impact of different FPSE parameters on extraction sensitivity: (A) sorbent coating chemistry; (B) sample volume; (C) extraction time; (D) stirring speed

tion trials. As such, magnetic stirring at a speed of 800 rpm was taken as optimum for all remaining experiments.

3.2.5 | Optimization of desorption solvent, volume and time

Selection of a suitable solvent/solvent system is an important parameter in FPSE that ensures quantitative recovery of the extracted analyte(s) from the FPSE media following the extraction. The effectiveness of three solvents, 100% methanol, (50:50 v/v) methanol/acetonitrile, and 100% acetonitrile, each at 500 µL volume, was investigated by comparing back extractions with each in triplicate. Due to the lower UV cut-off value of acetonitrile compared to methanol, 100% acetonitrile performed the best as the eluting solvent. FPSE media prepared with hydrophilic cotton cellulose inherently diffuse polar solvents through its body due to capillary action and therefore do not require any external stimuli during elution of the analyte. No significant difference was seen between 5 min and 10 min desorption. Therefore, 5 min was selected as the optimum desorption time. After the first desorption, subsequent back extraction did not reveal any residual signal of any of the six compounds, indicating that there was no carry-over.

3.2.6 | Establishing maximum analyte sorption capacity of the FPSE media

Sponge-like porous architecture of sol-gel PTHF sorbent coated as a thin film on 100% cellulose cotton fabric translates into high analyte retention capacity. A 2.5 × 2.0 cm FPSE media coated with sol-gel PTHF possesses 19.8 mg of sol-gel sorbent. To establish the maximum analyte retention capacity of sol-gel THF coated FPSE media, a series of whole milk samples were prepared at 20, 40, 60, 80, and 100 ppm concentrations of the six selected EDCs. The solubility of some of the six EDCs in milk ceased at 100 ppm and therefore no higher concentration could be tested. 5 mL of each of the solutions were analysed in triplicate under optimum extraction conditions. Extracted analytes were back-extracted in 500 µL of acetonitrile. FPSE-HPLC-UV data obtained for different concentration of EDCs are presented in Supporting Information Figure S4. The results unequivocally support that sol-gel PTHF coated FPSE media indeed possesses high analyte retention capacity for any practical application.

3.2.7 | Impact of fat content of milk on extraction sensitivity

Due to the presence of high mass of fat and protein in milk, samples often undergo a series of operations in order to eliminate/reduce the amount of fat and protein. These steps are time-consuming, tedious, and often lead to analyte loss and subsequently a lower reported value of the target pollutant. However, the classical sample preparation techniques require the removal of fat and proteins prior to analyte extraction. On the contrary, FPSE media can still perform well even if a high mass of fat and proteins are present in the sample. In the current study, whole milk (~3.25% fat), reduced fat milk (~2% fat), low fat milk (~1% fat), and skimmed milk (0–0.5%) were subjected to the validated analytical method. The SEM images of FPSE media captured before and after exposing to different milk samples under the optimum extraction conditions are presented in Figure 2. It is evident that some of the exposed surface of the FPSE media was covered by the fat molecules, with whole milk covering the most and skimmed milk the least. However, due to the strong hydrophilic nature

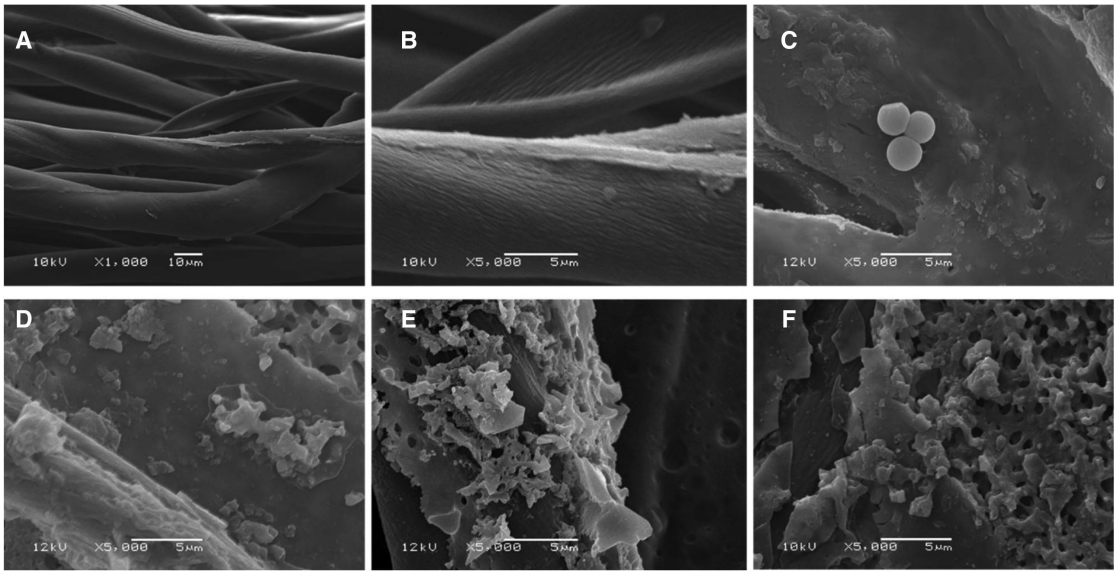

FIGURE 2 SEM images of sol-gel PTHF coated FPSE media: (A) before exposing to milk sample at 1000× magnifications; (B) before exposing to milk sample at 5000× magnifications; (C) after exposing to skimmed milk at 5000× magnifications; (D) after exposing to low-fat milk at 5000x magnifications; (E) after exposing to reduced-fat milk at 5000× magnifications; (F) after exposing to whole milk at 5000× magnifications

of both the substrate (100% cellulose) and the sol-gel PTHF coating, fat molecules did not cover the majority of the FPSE media. The analytical results presented in Supporting Information Figure S5 (a) reveal that extraction efficiency (in terms of arbitrary chromatographic peak area) is directly correlated to the fat content of the milk. The impact of current practice of protein precipitation on analyte extraction was also assessed using formic acid and acetic acid as protein precipitation agents. The data (presented in Supporting Information Figure S5 (b)) reveal that some of the analytes disappear during the protein precipitation step and subsequently may lead to a false negative report.

3.3 | Performance evaluations of FPSE-HPLC-UV detection method

3.3.1 | Selectivity

The high resolution between the selected EDCs (larger than baseline separation) and low background signal are indicative of good selectivity achieved by the new method. The sol-gel PTHF coated FPSE media was highly selective towards the target analytes as evidenced in the HPLC-UV and LC-MS/MS chromatograms (Supporting Information Figures S2 and S3). Although whole milk contains numerous compounds that may compete with the target analytes and co-extracted by the extracting sorbent, the cleaner chromatograms demonstrate that only the target analytes were preferentially extracted by the FPSE media.

3.3.2 | Linearity

Standard solutions prepared in whole milk (~3.25% fat content) showed linearity for BPA, α-E2, and E1 in the range of 25 ppb to 10 ppm. 17α-EE2, DES, and HEX showed linearity between 50 ppb and 20 ppm. Calibration curves for standard EDCs in whole milk are the following: $y = 64.312x$ ($R^2 = 0.9989$) for α-E2; $y = 56.649x$ ($R^2 = 0.9992$) for E1; $y = 103.41x$ ($R^2 = 0.9983$) for BPA; $y = 41.724x$ ($R^2 = 0.9992$) for 17α-EE2; $y = 46.206x$ ($R^2 = 0.9986$) for HEX and $y = 39.586x$ ($R^2 = 0.9985$) for DES.

3.3.3 | Accuracy and precision

The accuracy (% recovery) and precision (% RSD) of the FPSE-HPLC-UV method were evaluated for each of the six EDCs by analysing whole milk, 2% milk, 1% milk, and skimmed milk impregnated at 200, 300, and 500 ng/mL concentrations in triplicate. The results are presented in Table 1. As expected, the relative recovery values of the EDCs in different types of milk samples followed a general trend, higher recovery for a compound with lower logKow value (may be attributed to increased hydrophilicity) and lower recovery with higher logKow value (may be attributed to decreased hydrophilicity). The recovery values for all six EDCs increased with the decrease in the fat content of the milk sample. Although the recovery values are relatively low, the precision results show high reproducibility in the form of low % RSD values. As such, the proposed FPSE-HPLC-UV method not only eliminates the necessity for fat and protein removal from the milk samples, but also ensures high quality analytical data, an essential objective of quality assurance program to safeguard consumers' health and well-being. No internal standard was used in the current study due to the fact that it is highly unlikely that all six compounds would be found

TABLE 1 Relative recovery values obtained at different concentrations of the target analytes from different type of milk samples

Compound	Added concentration (ng/mL)	Relative recovery (%) (RSD, %; n = 3)			
		Whole Milk	2% Milk	1% Milk	Skimmed Milk
BPA	200	30.4 (11.9)	35.9 (12.5)	41.4 (8.2)	45.6 (5.1)
	300	29.7 (4.9)	35.8 (4.3)	39.8 (13.9)	44.2 (4.4)
	500	35.7 (3.6)	38.1 (2.8)	38.3 (6.1)	44.7 (6.9)
E2	200	28.2 (10.7)	34.0 (8.4)	57.0 (5.6)	69.2 (6.3)
	300	25.4 (7.7)	37.9 (7.5)	52.5 (10.2)	44.2 (4.4)
	500	37.6 (3.5)	40.3 (4.0)	47.4 (8.3)	58.4 (7.8)
17α-EE2	200	18.3 (4.1)	20.5 (9.3)	28.0 (16.0)	38.0 (3.4)
	300	16.5 (5.3)	21.7 (10.8)	25.0 (21.4)	34.6 (3.7)
	500	27.1 (12.9)	26.2 (3.2)	27.6 (8.2)	39.4 (9.1)
E1	200	21.4 (13.6)	34.9 (6.0)	50.2 (3.8)	59.3 (4.6)
	300	22.6 (7.0)	36.1 (3.5)	47.4 (10.6)	57.7 (2.1)
	500	35.9 (5.7)	38.4 (3.4)	43.5 (7.6)	56.9 (5.6)
DES	200	14.3 (10.0)	18.0 (8.7)	23.8 (5.8)	33.4 (1.8)
	300	16.2 (20.9)	17.8 (8.0)	25.8 (4.2)	37.8 (7.4)
	500	17.9 (5.2)	22.4 (8.0)	27.3 (2.7)	34.0 (8.9)
HEX	200	13.7 (6.4)	17.0 (2.2)	24.6 (9.5)	33.0 (3.4)
	300	14.7 (1.4)	17.3 (15.3)	24.6 (9.5)	32.7 (5.6)
	500	18.8 (16.1)	20.3 (4.0)	23.7 (2.6)	34.7 (10.5)

in routine milk analysis and any of these compounds can be used as the internal standard in routine quality monitoring.

3.3.4 | Repeatability and sensitivity

The repeatability of the FPSE-HPLC-UV method was investigated both intraday (n = 5) and interday (n = 3). The results, expressed in % RSD, for intra- and interday reproducibility, respectively, are given in Table 2.

The LOD and LOQ were determined for the FPSE-HPLC-UV method as representation of the sensitivity. These values were calculated such that the LOQ matches the low limit of the linearity curve. The LOD was deducted from the LOQ by dividing the LOQ by 10/3. Table 2 presents the LOD and LOQ values for each of the six EDCs in the study.

3.4 | Performance comparison with contemporary methods

The performance of the new FPSE–HPLC–UV method was compared with other reported methods in terms of LOQ values. The comparison data is included in Table 3. As the presented data reveals, the new methods offer similar or better LOQ values even though no protein precipitation, defatting, or dilution process were applied to the milk prior to the extraction. In addition, the commonly implemented solvent evaporation and sample reconstitution steps were eliminated from the sample preparation workflow. The major objective of the current approach was to simplify the entire analytical approach so that it can be readily adopted by the quality control laboratories of the milk producing enterprises as well as the regulatory agencies. Methods with lower LOQ values [18] applied protein precipitation, defatting, dilution, solvent evaporation, and sample reconstitution to gain method sensitivity advantage. However, these approaches are time consuming and considered as impractical in routine testing lab where high throughput analysis is a major priority. The sensitivity of the method can be easily enhanced multiple orders of magnitude by applying solvent evaporation and sample reconstitution. Since FPSE uses only 500 μL back-extraction solvent (can be even lower), solvent evaporation and sample reconstitution steps will not extend the overall sample preparation time as much as it does for other methodologies that require larger volumes of elution solvent. The advantage that can be achieved by adding solvent evaporation and sample reconstitution to the FPSE-HPLC-UV method has been illustrated in Supporting Information Figure S6. Figure 3 further illustrates the operational difference between FPSE, SBSE, and SPE by comparing the steps involved in each methodology. It is evident that FPSE considerably simplifies the sample preparation in terms of time, cost, and solvent consumption.

UHPLC-QTOF-MS [15] or UHPLC-MS/MS [6] seem to offer a huge advantage in analytical sensitivity as evident in Table 3. Such advantage can be easily exploited by combining FPSE with these techniques.

TABLE 2 Analytical figures of merit for the FPSE-HPLC-UV method

Compounds	Intra-day Repeatability (RSD,%)	Inter-day Repeatability (RSD,%)	Linearity Range (ng/mL)	Coefficient of Determination (R^2)	LOD (ng/mL)	LOQ (ng/mL)
BPA	3.6	12.8	25–10000	0.9983	7.5	25.0
α-E2	8.1	4.5	25–10000	0.9989	7.5	25.0
17α-EE2	8.9	9.3	50–20000	0.9992	7.5	50.0
E1	3.9	7.2	25–10000	0.9992	15.0	25.0
DES	13.9	8.8	50–20000	0.9985	7.5	50.0
HEX	8.6	11.3	50–20000	0.9986	15.0	50.0

TABLE 3 Comparison of the LOQ values obtained from FPSE-HPLC-UV with other reported methods

Compounds	FPSE-HPLC-UV (current study) LOQ (ng/mL)	HF-LPME-HPLC-DAD/FD[19] LOQ (ng/mL)	LLE-SPE-HPLC-DAD [17] MQL (ng/mL)	MME-SPME-HPLC-DAD [18] LOQ (ng/mL)	QuEChERS-dSPE-UHPLC-MS/MS [6] LOQ (ng/mL)	LLE/SPE/UPLC/QTOF-MS [15] LOQ (ng/mL)
BPA	25.0	N/A	N/A	0.31	N/A	0.01
E2	25.0	2.7	630	N/A	N/A	0.03
EE2	50.0	5.7	N/A	N/A	0.03	0.03
E1	25.0	43.3	360	N/A	0.02	0.04
DES	50.0	269	100	0.83	0.15	0.02
HEX	50.0	44.0	340	0.03	0.02	0.02

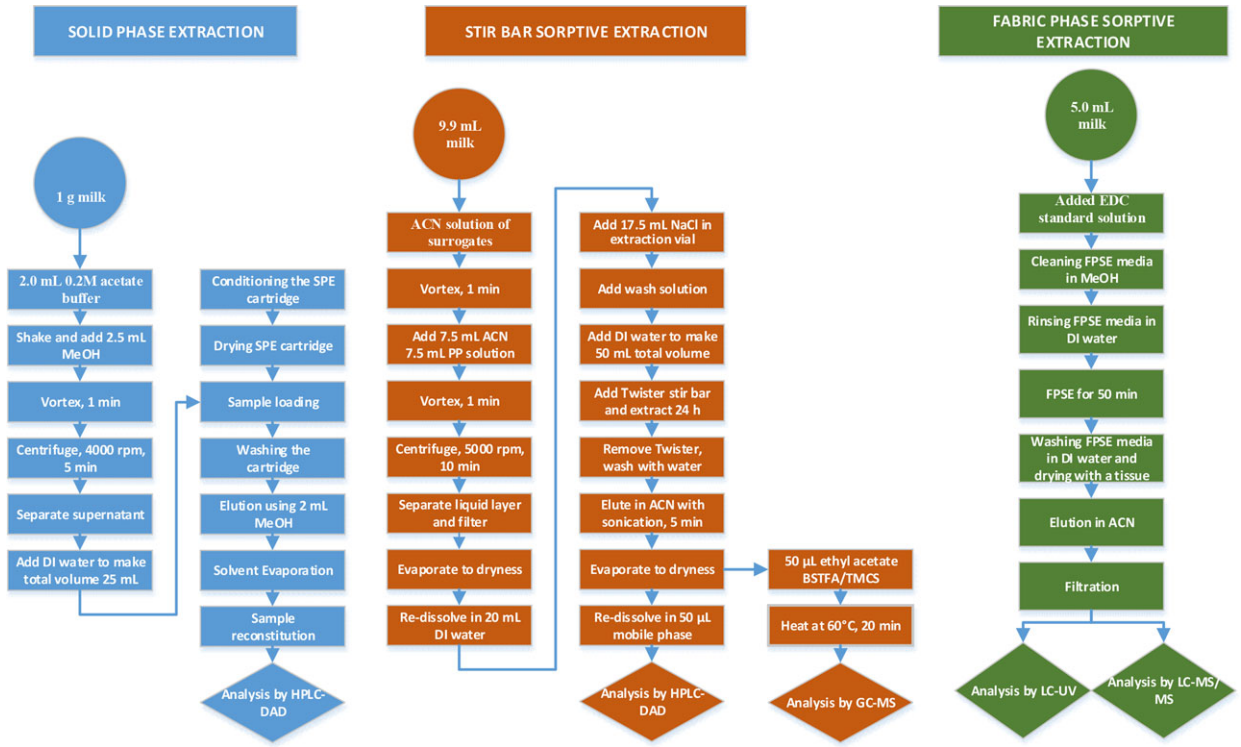

FIGURE 3 Comparison of milk sample preparation steps involved in SPE, SBSE and FPSE

3.5 | Confirmation of individual analyte identity LC-MS/MS

Due to the complexity of milk as an analytical sample matrix, it was necessary to confirm the identity of the chromatographic responses obtained in FPSE-HPLC-UV method. This cannot be done using only HPLC-UV instrumentation. As such, the FPSE sample preparation method was also coupled to LC-MS/MS in multiple reaction monitoring mode. Whole milk was spiked at a concentration of 10 ng/mL for each of the six EDCs in the study. The HPLC-UV and LC-MS/MS chromatograms are presented in Supporting Information Figures S2 and S3, respectively.

4 | CONCLUDING REMARKS

A simple, green, and robust analytical strategy is the key to ensure the quality of milk and other dairy products to safeguard consumers' health, safety, and well-being. FPSE has profoundly simplified the sample preparation workflow currently used in quality assurance of milk and has streamlined the current practice by eliminating protein precipitation and defatting steps prior to the extraction of target analytes. Additionally, elimination of post-treatment step such as solvent evaporation and sample reconstitution in FPSE is consistent with green analytical chemistry (GAC) principles. Simultaneous elimination of these time-consuming and error prone steps from the sample preparation workflow not only will help ensuring high quality analytical data, leading to better consumer confidence, but also will substantially reduce environmental pollution originated from solvent evaporation. FPSE of the target analytes from intact milk samples can be integrated with HPLC-UV or LC-MS, depending on the availability of chromatographic system as well as the analytical need. Sol-gel PTHF coating as the FPSE sorbent has demonstrated unique selectivity towards EDCs of medium and low polarity. While exhibiting competitively low LOD/LOQ values, excellent repeatability (RSD 3.6–13.9%), intermediate precision (RSD 4.6–12.7%), and broad linear range, the developed method positions itself as a readily deployable, robust chromatographic technique equally suitable for milk industries as well as the food quality monitoring agencies to assure milk quality in a routine manner.

ACKNOWLEDGMENTS

The authors are thankful to Mr. Thomas Beasley, Florida Center for Analytical Electron Microscopy at Florida International University for their help in obtaining SEM images.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ORCID

Abuzar Kabir https://orcid.org/0000-0002-3740-8857
Victoria Samanidou
https://orcid.org/0000-0002-8493-1106

REFERENCES

1. Locatelli, M., Sciascia, F., Cifelli, R., Malatesta, L., Bruni, P., Croce, F., Analytical methods for the endocrine disruptor compounds determination in environmental water samples. *J. Chromatogr. A* 2016, *1434*, 1–18.
2. Gañán, J., Morante-Zarcero, S., Pérez-Quintanilla, D., Marina, M. L., Sierra, I., One-pot synthesized functionalized mesoporous silica as a reversed-phase sorbent for solid-phase extraction of endocrine disrupting compounds in milks. *J. Chromatogr. A* 2016, *1428*, 228–235.
3. Juan-Garcia, A., Font, G., Pico, Y., Determination of quinolone residues in chicken and fish by capillary electrophoresis-mass spectrometry. *Electrophoresis* 2006, *27*, 2240–2249.
4. Samanidou, V., Evaggelopoulou, E., Trötzmüller, M., Guo, X., Lankmayr, E., Multi-residue determination of seven quinolones antibiotics in gilthead seabream using liquid chromatography–tandem mass spectrometry. *J. Chromatogr. A* 2008, *1203*, 115–123.
5. Niu, Y., Zhang, J., Wu, Y., Shao, B., Analysis of bisphenol A and alkylphenols in cereals by automated on-line solid-phase extraction and liquid chromatography tandem mass spectrometry. *J. Agric. Food Chem*. 2012, *60*, 6116–6122.
6. Malone, E. M., Elliott, C. T., Kennedy, D. G., Regan, L., Rapid confirmatory method for the determination of sixteen synthetic growth promoters and bisphenol A in bovine milk using dispersive solid-phase extraction and liquid chromatography–tandem mass spectrometry. *J. Chromatogr. B* 2010, *878*, 1077–1084.
7. Huang, Z., Ma, J., Chen, B., Zhang, Y., Yao, S., Determination of cyclamate in foods by high performance liquid chromatography–electrospray ionization mass spectrometry. *Anal. Chim. Acta* 2006, *555*, 233–237.
8. Huang, X., Qiu, N., Yuan, D., Simple and sensitive monitoring of sulfonamide veterinary residues in milk by stir bar sorptive extraction based on monolithic material and high performance liquid chromatography analysis. *J. Chromatogr. A* 2009, *1216*, 8240–8245.
9. Li, J., Chen, H., Chen, H., Ye, Y., Selective determination of trace thiamphenicol in milk and honey by molecularly imprinted polymer monolith microextraction and high-performance liquid chromatography. *J. Sep. Sci.* 2012, *35*, 137–144.
10. Shi, X., Wu, A., Zheng, S., Li, R., Zhang, D., Molecularly imprinted polymer microspheres for solid-phase extraction of chloramphenicol residues in foods. *J. Chromatogr. B* 2007, *850*, 24–30.
11. Xu, W., Su, S., Jiang, P., Wang, H., Dong, X., Zhang, M., Determination of sulfonamides in bovine milk with column-switching high performance liquid chromatography using surface imprinted silica with hydrophilic external layer as restricted access and selective extraction material. *J. Chromatogr. A* 2010, *1217*, 7198–7207.
12. Nicolich, R. S., Werneck-Barroso, E., Marques, M. A. S., Food safety evaluation: detection and confirmation of chloramphenicol in milk by high performance liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* 2006, *565*, 97–102.
13. Nebot, C., Regal, P., Miranda, J. M., Fente, C., Cepeda, A., Rapid method for quantification of nine sulfonamides in bovine milk using HPLC/MS/MS and without using SPE. *Food Chem*. 2013, *141*, 2294–2299.
14. Tolika, E. P., Samanidou, V. F., Papadoyannis, I. N., Development and validation of an HPLC method for the determination of ten sulfonamide residues in milk according to 2002/657/EC. *J. Sep. Sci.* 2011, *34*, 1627–1635.
15. Wang, H.-X., Zhou, Y., Jiang, Q.-W., Enhanced screening efficiency for endocrine-disrupting chemicals in milk and powdered milk using UPLC/QTOF-MS by the introduction of dansyl chloride derivatisation. *Food Addit. Contam. Part A* 2013, *30*, 166–180.
16. Karageorgou, E. G., Samanidou, V. F., Development and validation according to European Union Decision 2002/657/EC of an

HPLC-DAD method for milk multi-residue analysis of penicillins and amphenicols based on dispersive extraction by QuEChERS in MSPD format. *J. Sep. Sci.* 2011, *34*, 1893–1901.

17. Yan, W., Li, Y., Zhao, L., Lin, J.-M., Determination of estrogens and bisphenol A in bovine milk by automated on-line C30 solid-phase extraction coupled with high-performance liquid chromatography–mass spectrometry. *J. Chromatogr. A* 2009, *1216*, 7539–7545.
18. Mei, M., Yu, J., Huang, X., Li, H., Lin, L., Yuan, D., Monitoring of selected estrogen mimics in complicated samples using polymeric ionic liquid-based multiple monolithic fiber solid-phase microextraction combined with high-performance liquid chromatography. *J. Chromatogr. A* 2015, *1385*, 12–19.
19. Socas-Rodríguez, B., Asensio-Ramos, M., Hernández-Borges, J., Rodríguez-Delgado, M. Á., Hollow-fiber liquid-phase microextraction for the determination of natural and synthetic estrogens in milk samples. *J. Chromatogr. A* 2013, *1313*, 175–184.
20. Holstege, D. M., Puschner, B., Whitehead, G., Galey, F. D., Screening and mass spectral confirmation of β-lactam antibiotic residues in milk using LC-MS/MS. *J. Agric. Food Chem.* 2002, *50*, 406−411.
21. Wang, L., Li, Y.-Q., Simultaneous determination of ten antibiotic residues in milk by UPLC. *Chromatographia* 2009, *70*, 253–258.
22. Buszewski, B., Szultka, M., Past, present, and future of solid phase extraction: a review. *Crit. Rev. Anal. Chem.* 2012, *42*, 198–213.
23. Kabir, A., Mesa, R., Jurmain, J., Furton, K. G., Fabric phase sorptive extraction explained. *Separations* 2017, *4*, 21.
24. Kabir, A., Furton, K. G., Fabric phase sorptive extractors. U.S. Patent 9,283,544, March 15, 2016.
25. Karageorgou, E., Manousi, N., Samanidou, V., Kabir, A., Furton, K. G., Fabric phase sorptive extraction for the fast isolation of sulfonamides residues from raw milk followed by high performance liquid chromatography with ultraviolet detection. *Food Chem*. 2016, *196*, 428–436.
26. Samanidou, V., Filippou, O., Marinou, E., Kabir, A., Furton, K. G.. Sol–gel-graphene-based fabric-phase sorptive extraction for cow and human breast milk sample cleanup for screening bisphenol A and residual dental restorative material before analysis by HPLC with diode array detection. *J. Sep. Sci.* 2017, *40*, 2612–2619.
27. Samanidou, V., Galanopoulos, L.-D., Kabir, A., Furton, K. G., Fast extraction of amphenicols residues from raw milk using novel fabric phase sorptive extraction followed by high-performance liquid chromatography-diode array detection. *Anal. Chim. Acta* 2015, *855*, 41–50.
28. Samanidou, V., Kabir, A., Furton, K. G., Michaelidou, K., Fabric phase sorptive extraction of selected penicillin antibiotic residues from intact milk followed by high performance liquid chromatography with diode array detection. *Food Chem*. 2017, *224*, 131–138.
29. Locatelli, M., Tinary, N., Grassadonia, A., Tartaglia, A., Macerola, D., Piccolantonio, S., Sperandio, E., D'Ovidio, C., Carradori, S., Ulusoy, H. I., Furton, K. G., Kabir, A., FPSE-HPLC-DAD method for the quantification of anticancer drugs in human whole blood, plasma, and urine. *J. Chromatogr. B* 2018, *1095*, 204–213.
30. Kabir, A., Furton, K. G., Tinary, N., Grossi, L., Innosa, D., Macerola, D., Tartaglia, A., Donato, V. D., D'Ovidio, C., Locatelli, M., Fabric phase sorptive extraction-high performance liquid chromatography-photo diode array detection method for simultaneous monitoring of three inflammatory bowel disease treatment drugs in whole blood, plasma and urine. *J. Chromatogr. B* 2018, *1084*, 53–63.
31. Locatelli, M., Kabir, A., Innosa, D., Lopatriello, T., Furton, K. G., A Fabric phase sorptive extraction-high performance liquid chromatography-photo diode array detection method for the determination of twelve azole antimicrobial drug residues in human plasma and urine. *J. Chromatogr. B* 2017, *1040*, 192–198.
32. Kaklamanos, G., Theodoridis, G., Rapid multi-method for the determination of growth promoters in bovine milk by liquid chromatography–tandem mass spectrometry. *J. Chromatogr. B* 2013, *930*, 22–29.
33. Tso, J., Aga, D. S. A., systematic investigation to optimize simultaneous extraction and liquid chromatography tandem mass spectrometry analysis of estrogens and their conjugated metabolites in milk. *J. Chromatogr. A* 2010, *1217*, 4784–4795.
34. Azzouz, A., Jurado-Sánchez, B., Souhail, B., Ballesteros, E., Simultaneous determination of 20 pharmacologically active substances in cow's milk, goat's milk, and human breast milk by gas chromatography–mass spectrometry. *J. Agric. Food Chem.* 2011, *59*, 5125–5132.
35. Azner, M., Alfaro, P., Nerin, C., Kabir, A., Furton, K. G., Fabric phase sorptive extraction: an innovative sample preparation approach applied to the analysis of specific migration from food packaging. *Anal. Chim. Acta* 2016, *936*, 97–107.
36. Kabir, A., Furton, K. G., Malik, A.. Innovations in sol-gel microextraction phases for solvent-free sample preparation in analytical chemistry. *TrAC Trends Anal. Chem.* 2013, *45*, 197–218.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Mesa R, Kabir A, Samanidou V, Furton KG. Simultaneous determination of selected estrogenic endocrine disrupting chemicals and bisphenol A residues in whole milk using fabric phase sorptive extraction coupled to HPLC-UV detection and LC-MS/MS. *J Sep Sci* 2018;1–11. https://doi.org/10.1002/jssc.201800901