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A Facile Fabric Phase Sorptive Extraction Method for Monitoring Chloramphenicol Residues in Milk Samples

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Abstract:	Determination of pharmaceutical elements and pharmacologically active molecules in the biological matrices is crucial in various fields of clinical and pharmaceutical chemistry, e.g., in pharmacokinetic studies, developing new drugs, or therapeutic drug monitoring. Chloramphenicol (CP) is used for treating bacterial infections, and it's one of the first antibiotics synthetically manufactured on a large scale. Fabric phase sorptive extraction (FPSE) was used to determine Chloramphenicol antibiotic residues in milk samples by means of validated HPLC-DAD instrumentation. Cellulose fabric phases modified with polyethylene glycol-block-polypropylene glycol-block-polyethylene glycol triblock copolymer was synthesized using sol-gel synthesis approach (Sol-gel PEG-PPG-PEG) and used for batch-type fabric phase extractions. Experimental variables of the FPSE method for antibiotic molecules were investigated and optimized systematically. The HPLC analysis of chloramphenicol was performed using a C18 column, isocratic elution of trifluoroacetic acid (0.1%), methanol, and acetonitrile (17:53:30) with a flow rate of 1.0 mL/min. The linear range for the proposed method for chloramphenicol (r2 > 0.9982) was obtained in the range of 25.0–1000.0 ng/mL. The limit of detections (LOD) is 8.3 ng/mL, while RSDs% are below 4.1%. Finally, the developed method based on FPSE-HPLC-DAD was applied to milk samples to quantitatively determine antibiotic residues.
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Response to Reviewers:	We thank, the Reviewer for his/her useful comments and careful reading of the manuscript. We have modified the manuscript as per comments and addressed all comments raised by the reviewers. Below, written in red, a detailed reply and/or rebuttal to all comments raised by reviewer. Reviewer #2: Reviewer #2: The authors still did not include the milk calibration strategy in the manuscript. I am not asking for convincement via more references from the literature. I am only suggesting them to add this information to the manuscript so any future reader have an idea how it was done in this study. I am suggesting the following information written by the authors (as reply to reviewer) to be added to the related part of the manuscript: 'Briefly, the FPSE method was developed by model solutions and milk samples submitted to developed method after a

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Amina Ben Ayed
Sfax, Tunisia
December 2023

Dear Editors,

Manuscript Number: JPBA-D-23-01763R2

Manuscript title: A Facile Fabric Phase Sorptive Extraction Method for Monitoring Chloramphenicol Residues in Milk Sample

First of all, we are thankful for showing interest in our work and we would like to thank you as well for your valuable remarks and constructive comments and suggestions raised by the editor and the reviewers of our manuscript (Ref. No.: JPBA-D-23-01763R2) entitled: " A Facile Fabric Phase Sorptive Extraction Method for Monitoring Chloramphenicol Residues in Milk Sample".

We greatly appreciate your kind request to revise our manuscript in the light of those constructive and insightful peer review comments. We have replied to the comments of reviewer 2. We would therefore be pleased to resubmit a revised version of our improved manuscript which addresses all concerns and requests. For the convenience of reviewing, we have highlighted the texts that have been modified/revised in the revised version with red color.

We would be glad if you could consider our revised manuscript for possible publication in the Journal of Pharmaceutical and Biomedical Analysis.

On behalf of all co-authors,

Please address all correspondence concerning this manuscript to me at:

benayed.aminalfpc@gmail.com

hiulusoy@yahoo.com

Thank you for your consideration of this manuscript.

Sincerely yours,

Amina Ben ayed

Reply to reviewers

We thank, the Reviewer for his/her useful comments and careful reading of the manuscript. We have modified the manuscript as per comments and addressed all comments raised by the reviewers. Below, written in red, a detailed reply and/or rebuttal to all comments raised by reviewer.

Reviewer #2: Reviewer #2: The authors still did not include the milk calibration strategy in the manuscript. I am not asking for convincement via more references from the literature. I am only suggesting them to add this information to the manuscript so any future reader have an idea how it was done in this study.

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Prof.Dr. Halil İbrahim Ulusoy

Highlights

- FPSE Method for Monitoring Chloramphenicol Residues in Milk Samples
- Pharmaceuticals and active molecules in biological matrices determination
- Chloramphenicol quantitative analysis in milk samples

Authorship contribution statement

Amina Ben Ayed: Investigation, Writing – original draft. **Halil İbrahim Ulusoy**: Data curation, Supervision, Project administration, Funding acquisition **Ümmügülsüm Polat:** Formal analysis. **Songül Ulusoy**: Conceptualization, Validation. **Marcello Locatelli**: Writing – original draft. **Abuzar Kabir:** Synthesis and Characterization, Data curation, Validation. **Hamadi Khemakhem**: Writing – review & editing.

A Facile Fabric Phase Sorptive Extraction Method for Monitoring Chloramphenicol Residues in Milk Samples

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Abstract

Determination of pharmaceutical elements and pharmacologically active molecules in the biological matrices is crucial in various fields of clinical and pharmaceutical chemistry, e.g., in pharmacokinetic studies, developing new drugs, or therapeutic drug monitoring. Chloramphenicol (CP) is used for treating bacterial infections, and it's one of the first antibiotics synthetically manufactured on a large scale. Fabric phase sorptive extraction (FPSE) was used to determine Chloramphenicol antibiotic residues in milk samples by means of validated HPLC-DAD instrumentation. Cellulose fabric phases modified with polyethylene glycol-block-polypropylene glycol-block-polyethylene glycol triblock copolymer was synthesized using sol-gel synthesis approach (Sol-gel PEG-PPG-PEG) and used for batch-type fabric phase extractions. Experimental variables of the FPSE method for antibiotic molecules were investigated and optimized systematically. The HPLC analysis of chloramphenicol was performed using a C18 column, isocratic elution of trifluoroacetic acid (0.1%), methanol, and acetonitrile (17:53:30) with a flow rate of 1.0 mL/min. The linear range for the proposed method for chloramphenicol ($r^2 > 0.9982$) was obtained in the range of 25.0–1000.0 ng/mL. The limit of detections (LOD) is 8.3 ng/mL, while RSDs% are below 4.1%. Finally, the developed method based on FPSE-HPLC-DAD was applied to milk samples to quantitatively determine antibiotic residues.

Keywords: Chloramphenicol; Fabric phase sorptive extraction; HPLC; Milk samples.

1. Introduction

The demand for food and other resources is rising as a result of the growing world population and shifting standards of life. Particularly, the need for healthy, safe food is rising, and the dairy industry has greatly benefited from this demand. Since ancient times, milk has been revered as nature's ideal diet and is a widely consumed commodity. It is a great source of nutrients and has the right proportions of proteins, lipids, carbs, vitamins, and minerals, which offers a number of advantages for development, immunity, and growth. With more than 6 billion customers worldwide, milk and milk products also contribute significantly to the growth of the food sector and economy. The presence of veterinary drug residues in milk, such as antibiotics, diminishes its nutritional value largely. Some scientists have found that the chemical residues in milk may contain medications used in veterinary therapies or from cleaning and other industrial activities in livestock facilities [1]. As a result, the quality of dairy products may be impacted by the presence of a wide range of organic chemical substances, including drugs, surfactants, and disinfectants. Due to antibiotic residues in foods of animal origin and water sources, humans are most impacted by poor cell membrane permeability, myalgia, skin rash, tendon rupture, hyperactivity, cancer, gastrointestinal and cardiovascular illnesses, etc.[2]. Antibiotics are heavily present in humans, animals, the environment, and food as a result of their widespread usage, low manufacturing costs, overuse, and abuse in the pharmaceutical and animal husbandry sectors of the economy. Because of this, certain microbes have become resistant to antibiotics [3]. Bacteria can proliferate and endure even in the presence of therapeutic dosages of antibiotics due to the adaptive genetic phenomena known as antibiotic resistance. Expressly, chloramphenicol (CP), also known as 2,2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl] acetamide, was discovered in 1947, in Streptomyces, Venezuela. The Parke-Davis team of researchers discovered and synthesized its molecular structure after two years in 1949 [4]. Chloramphenicol has been used to treat bacterial infections in people and animals. Additionally, CP is widely used in domestic poultry and animal feed production as a therapeutic and preventative agent against bacteria, for superficial eye infections, aquaculture, and beekeeping. This is owing to its low cost and great efficacy. CP misuse, on the other hand, has led to the entry of residues from animal products into the human body through the food chain and is the root of several chronic illnesses as cardiovascular failure, leukemia, and aplastic anemia. In **Table S1** (*Supplementary material*) were reported the main properties of CP. As a result, to maintain the safety of food items, the European Commission has set CP's minimum required performance limit (MRPL) at 0.3 µg/kg [5]. Thus, it is crucial to provide a quick and highly accurate approach for the monitoring and detection of CP in samples from various

clinical, environmental, and pharmacological sources. For the purpose of determining CP, a variety of analytical techniques and approaches have been created such as high-performance liquid chromatography (HPLC) [6], liquid chromatography-mass spectrometry (LC-MS) [7], gas chromatography-mass spectrometry (GC-MS) [8], gas chromatography (GC) [10], chemiluminescence [10] and capillary electrophoresis (CE) [11]. Due to the presence of these antibiotics in trace amounts in complex food matrices and the disruptive effects of matrix components, pre-separation and pre-concentration procedures are now required before these antibiotics are analyzed [12]. For the isolation and extraction of chloramphenicol, several sample classical techniques have been proposed such as the using of the salt-assisted liquid-liquid microextraction [13], magnetic solid phase extraction (MSPE) [12], solid-phase microextraction (SPME) [14], dual solid-phase microextraction [15], solid-phase extraction (SPE) [16], liquid-liquid extraction (LLE) [17]. Following the requirements of green analytical chemistry, modern analytical chemistry is now moving toward miniaturizing sample preparation and utilizing small quantities of organic solvent (or replace it). Additionally, several procedures require solvent evaporation and/or protein precipitation before the extraction, bringing to a significant loss of analytes. It is crucial to create sample preparation method that is simple, quick, efficient, and affordable, has a high analyte retention capacity, and permits regeneration for reuse of the same sorbent. For the above reasons, various novel extraction procedures that need less sample preparation and little sample volume have been developed recently.

In 2014, Kabir and Furton [18] created the fabric-phase sorptive extraction (FPSE), a modern sample preparation method, a cutting-edge sample preparation technique, combines the extraction mechanism of SPE and SPME into a single sample preparation platform. The FPSE provides a number of benefits such as it does not require sample pre-treatment procedures, such as filtration or centrifugation (or any other kind of pre-treatment procedure). In FPSE, a natural or synthetic fabric substrate is chemically treated to generate an ultra-thin coating of a hybrid sol-gel and organic-inorganic sorbent. A thin sorbent layer is formed during the chemically controlled sorbent coating process and then chemically bonds to the substrate surface. High porosity and easy permeability characterize the sol-gel sorbent-coated FPSE membrane. The use of FPSE has been reported earlier in numerous works for the extraction of developing pollutants, such as Venlafaxine [19], penicillin antibiotics [20], sulfonamides [21], amphenicols [22] and other compounds from raw or unprocessed milk with the least amount of sample preparation. Sensitive and selective findings for all types of samples may be produced using this approach in conjunction with a reliable separation and detection instrument.

The scope of the present research is to design and optimize for first time, a new analytical approach using sol–gel poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol)-modified cellulose fabric phase sorptive extraction (FPSE) membrane was exploited for the analysis of selected chloramphenicol antibiotic (CP) in milk samples prior to HPLC-DAD technique. Significant parameters such as the extraction time, sample volume and pH and elution solvent that influence the extraction effectiveness were carefully investigated. The adsorbent demonstrated good material performance, indicating that it might be exploited as a potential material in the development of FPSE technique with high analytical performance and it has effectively applied to the determination of the selected drugs in milk samples.

2. Materials and method

2.1 Chemicals and materials

The FPSE membranes used in this work were fabricated at the Department of Chemistry and Biochemistry, located in Florida International University, Miami, Florida, USA. In order to achieve the greatest quality possible, all chemicals, reagents, organic polymers, solvents and sol-gel precursor employed in the project were sourced from reputable commercial suppliers and ensured the highest quality available. Specifically, Methyltrimethoxysilane (MTMOS), trifluoroacetic acid (TFA), acetone, poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) polymer, and dichloromethane were obtained from Sigma-Aldrich, based in St. Louis, MO, USA. Additionally, muslin cotton fabric consisting of 100% cellulose was purchased from Jo-Ann Fabric in Miami, FL, USA. In the present project, all compounds employed were of high purity, with a minimum purity level of 99.5%. Deionized water with a resistivity of 18.2 MΩ cm, obtained from a MES Minipure Dest Up water purification system located in Ankara, Turkey, was used in all experimentations. For HPLC-DAD analysis, acetonitrile (ACN), methanol (MeOH), and trifluoroacetic acid (TFA) of HPLC-grade were utilized, sourced from Merck in Darmstadt, Germany. A stock solution of chloramphenicol at a concentration of 100 µg/mL (purchased from Sigma-Aldrich) was prepared using analytically pure methanol from Sigma Aldrich in St. Louis, MO, USA. Milk samples were obtained from local food stores in Sivas, Turkey, and various types of analyzed milk were stored refrigerated at a temperature of +4°C.

2.2 Instrumentation

An Eppendorf Centrifuge 5415 R (Eppendorf North America Inc., Hauppauge, NY, USA) was used to eliminate unwanted and interfering microparticles from the solutions prior to sol-

gel coating on the fabric substrate used to create FPSE membrane. For obtaining scanning electron microscope (SEM) images, a Philips XL30 Scanning Electron Microscope equipped with an EDAX detector was employed in this study. The SEM allowed for detailed visualization and analysis of the samples.

A Hettich Centrifuge (Universal 320, Hettich Lab Technology) was used to centrifugate various solutions in order to produce particle-free solutions. The full blending of several solutions was accomplished using a Fisher Scientific Digital shaker (Fisher brand) and a vortex mixer (Velp Scientifica F20220176 ZX3).

The pH readings were determined using a glass-calomel electrode pH meter (Mettler Toledo, Columbus, Ohio, OH, USA). For sample preparation, an ultrasonic water bath (made by Kudos, China) was employed. Prior to analysis, all chromatographic system solvents were degassed for 10 min in an ultrasonic bath (JP Selecta, Barcelona, Spain) and filtered through a 0.45 µm PTFE membrane filter (HNWP, Millipore) using a vacuum pump (Buchi, Switzerland).

2.3 Chromatographic analysis

The chromatographic setup used was equipped with a PDA detector SPD-M20A, an auto sampler SIL-20Ac, a thermostatic oven CTO-10 AS, and a pump type LC20-AD from Shimadzu, Tokyo, Japan (Shimadzu). LC solution software was used to transmit the obtained data to the computer (Shimadzu).

The HPLC analysis of CP was performed using a C18 column (Luna Omega C18, 250 mm x 4.6 mm, 5.0 µm) in isocratic elution mode using trifluoroacetic acid (0.1%), methanol, and acetonitrile (17:53:30, *v:v:v*) with a flow rate of 1.0 mL/min. The column was maintained at 40°C, while for quantitative analyses the detector wavelength was set at 276 nm. The injection volume was 10 µL. Prior to analysis, samples and mobile phases were ultrasonically degassed for 10 min and filtered using a membrane filter (0.45 µm). By comparing retention times and UV/Vis spectra of standards, each peak in the chromatograms was recognized. Analytical results were quantified by peak area at the respective analyte maximum wavelengths. Additional information about the HPLC configuration and setting were reported in **section S2** (*supplementary material*).

2.4 Preparation of Sol-gel PEG-PPG-PEG coated FPSE membrane

Commercial 100% cellulose cotton fabric was selected as the substrate for FPSE membrane. To remove any potential residual chemicals, present in the commercial source of the fabric and

to maximize the surface hydroxyl groups of the cellulose fabric, the fabric substrate was treated with 1M NaOH solution for 1h followed by rinsing with deionized water several times and subsequently treated with 0.1M HCl to neutralize any remnant of NaOH potentially present in the fabric substrate. The detail procedure of the fabric substrate treatment is presented elsewhere [23,24].

In order to ensure maximum extraction efficiency for the target analyte, a medium polarity sorbent, sol-gel PEG-PPG-PEG was designed and synthesized. The sol solution for creating the thin layer coating on the substrate surface was composed of an organically modified inorganic precursor, methyl trimethoxysilane (MTMS), an organic polymer, PEG-PPG-PEG, a solvent system (50:50, *v:v* methylene chloride: acetone), an acid catalyst (trifluoroacetic acid, TFA), and water. The molar ratio between the sol-gel precursor, organic/inorganic polymer, acetone, methylene chloride, TFA and water was optimized. The optimum molar ratio of the building blocks was maintained at: 1: 0.1: 3.26: 3.74: 1.25: 3 The detail process for preparing the sol solution and the sol-gel sorbent coating process via immersion-coating technology are presented elsewhere [24–26].

Briefly, building blocks of the individual sol solution were added sequentially into 50 mL high-density polyethylene centrifuge tube followed by vortexing for 3 min. Finally, the sol solution was centrifuged at 14000 rpm to remove any particulate matter suspended in the solution. The supernatant of the sol solution was then transferred in 50 mL amber glass reaction vessel and a pre-treated fabric (30 cm x 20 cm) was gently inserted in the sol solution to initiate the immersion coating process. The sol-gel sorbent coating continued for 6h at room temperature. Subsequently, the fabric substrate was removed from the sol-gel reaction vessel and air-dried for 1h. The sol-gel sorbent-coated FPSE membrane was then subjected to thermal conditioning in an inert environment at 50°C for 24h under continuous helium gas flow. The FPSE membrane was then rinsed with 50:50 (*v:v*) methylene chloride: methanol, air dried for 1h and thermally conditioned at 50°C for 8h. The FPSE membrane was cut into 1.0 cm x 1.0 cm units and stored in airtight container until their use in FPSE experiments.

2.5 FPSE procedure

The FPSE membrane was cleaned by immersing it in 2 mL of acetonitrile: methanol (50:50, *v:v*) for 5 min. No vortexing, shaking, or stirring was needed since the organic solvent combination may quickly penetrate through the porous sol-gel coating and permeable substrate matrix. The remaining organic solvents were washed from the FPSE membrane using 2 mL of deionized water. 2.0 mL of pH 7 buffer and 1 cm^2 of the fabric phase were added to 20 mL of

sample solution containing chloramphenicol in the range of 25-1000 ng/mL, and the final volume was raised to 50 mL with deionized water. After that, an orbital shaker was used to increase interactions between fabric phase and drug molecules for 50 min at 50 rpm. Each FPSE membrane was taken out and then, using 400 µL of methanol, the chloramphenicol was back-extracted. Vortex provided assistance with back-extraction for 20s. Finally, 0.45 µm membrane filters were used to filter the back-extracted solutions before the HPLC analysis.

2.6 Preparation of milk samples

To evaluate the effectiveness of the developed technique, real milk samples were employed. The milk samples underwent a straightforward procedure with minor modifications, as outlined in a previously published article [27]. In this process, 5 mL of homogenized milk was dropped into a 50 mL centrifuge tube. Subsequently, it was mixed vigorously using a vortex with 0.5 mL of 0.50% (*w:w*) ascorbic acid and 2 mL of methanol. The mixture was then shaken for 1 min using a vortex mixer. Following this, the samples were subjected to centrifugation at 3000 rpm for 5 min. The resulting supernatant phase was filtered through a 0.45 µm membrane filter. Finally, 2 mL of the filtered solution was utilized for fabric phase sorptive extraction using the developed method.

3. Results and discussion

3.1 Mechanism of extraction on the FPSE membrane

Major analytical challenges appear when the target analyte is relatively polar and dispersed in a complex sample matrix. Milk contains numerous matrix interferents including proteins, fats, salts, and many others. Sol-gel derived sorbent in FPSE, unlike sorbents in classical extraction and microextraction techniques, extracts analyte from the sample matrix by exerting different intermolecular interaction including London dispersion, hydrophobic/hydrophilic surface properties, dipole-dipole interactions, and hydrogen bonding towards the target analytes. The primary extraction mechanism of sol-gel PEG-PPG-PEG sorbent-coated FPSE membrane is governed by the adsorption of target analytes onto the surface of the sponge-like porous sol-gel sorbent via intermolecular interactions between the sorbent and the analytes. The sponge-like porous morphology of the sol-gel sorbent allows rapid permeation of the aqueous sample carrying the target analytes for interacting with the sorbent, resulting in fast extraction kinetic and short overall sample preparation time. Chloramphenicol is a medium polar analyte with a log Kow value of 1.14. A rational polymer selection for this analyte leads to medium polar polymer PEG-PPG-PEG. Due to the polymer segment connected as blocks, this unique

polymer exerts affinity towards polar and medium polar analytes very efficiently via different intermolecular interactions, resulting in high extraction efficiency for a wide range of analytes. As expected, the sol-gel PEG-PPG-PEG sorbent-coated FPSE membrane demonstrated very high affinity towards chloramphenicol as manifested by low limit of detection compared to other published analytical methods developed for chloramphenicol.

3.2 Characterization of sol–gel PEG-PPG-PEG coated FPSE membrane

3.2.1 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine the FPSE membrane's surface morphology. FPSE media exploit a number of merits offered by sol-gel coating technology, an extremely regulated surface coating method that ensures unrivaled coating homogeneity and chemical bonding between the sol-gel sorbent and the substrate. FPSE links solid phase extraction (characterized by exhaustive principle) and the extraction principles of solid phase microextraction (characterized by equilibrium extraction) by its unique design. In order to apply the concept of exhaustive extraction, the FPSE membrane must be permeable. As shown in **Figure 1 (a)**, cotton fabrics (100% cellulose) are designed to have good ventilation. **Figure 1 (b)** shows the surface morphology of the uncoated cellulose fabric at a magnification of 500x to show the individual microfibrils, which serve as the foundation for woven fabric, have a smooth, seemingly polished surface. SEM image of the sol-gel PEG-PPG-PEG coated FPSE membrane is shown in **Figure 1(c)** at 100x magnification. As can be seen, the through-holes in the FPSE membrane are still present, even after sol-gel coating of the sorbent. The benefit and ease of the sol-gel sorbent coating technique is demonstrated by a consistent coating surrounding each microfibril of the cellulose fabric as seen in **Figure 1 (d)** at 500x magnifications.

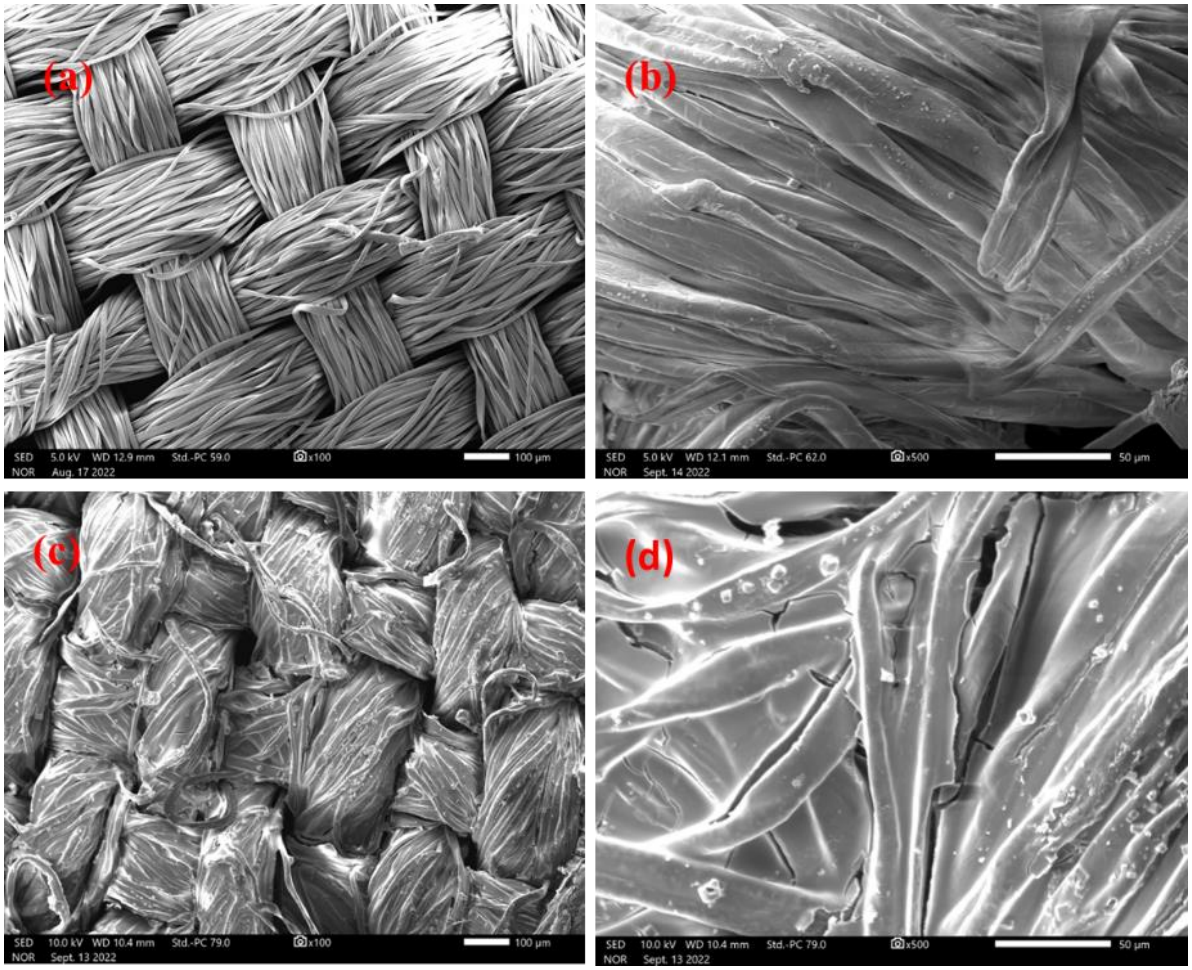
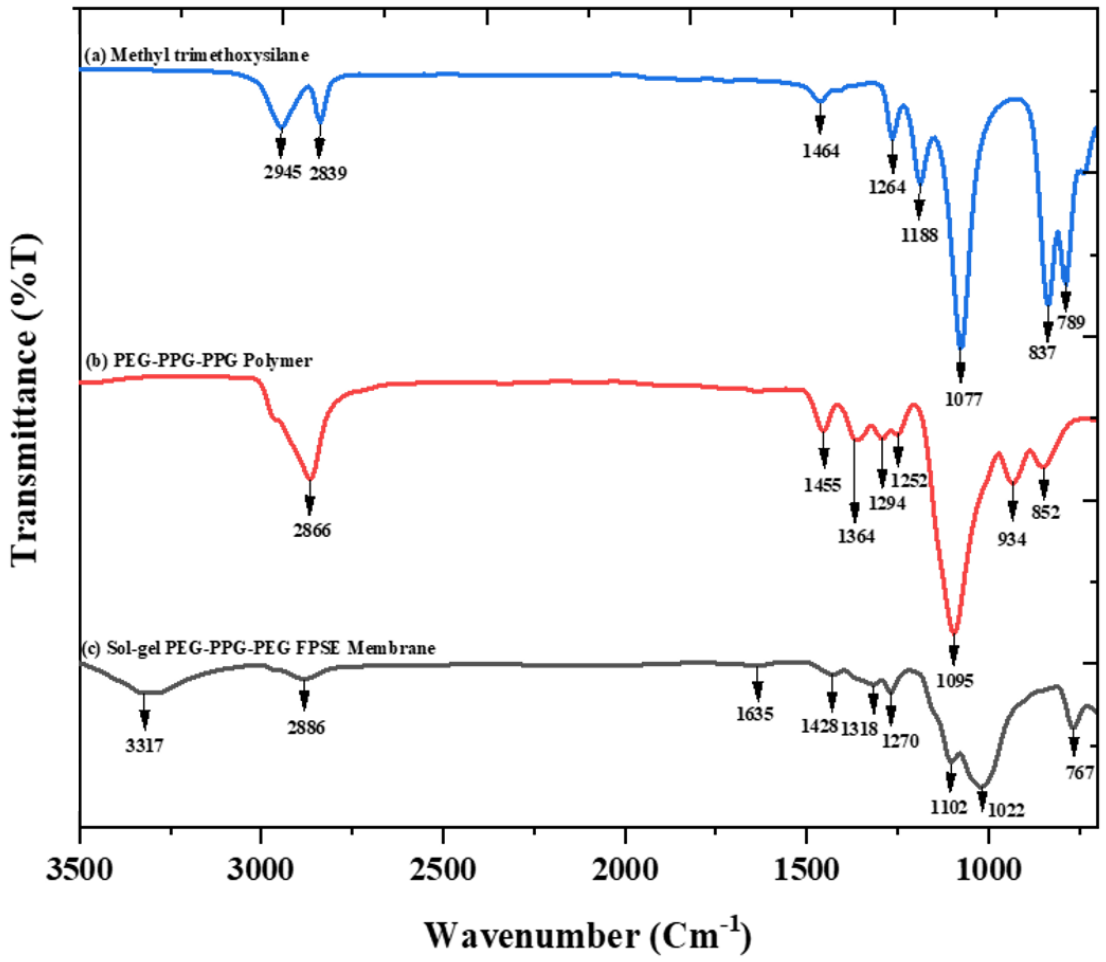


Figure 1. SEM images of (a) uncoated cellulose cotton fabric surface at 100x magnifications; (b) uncoated cellulose fabric surface at 500x magnifications; (c) sol-gel PEG-PPG-PEG coated cellulose fabric surface at 100x magnifications; (d) sol-gel PEG-PPG-PEG coated cellulose fabric surface at 500x magnifications.

3.2.2 Fourier Transform Infrared Spectroscopy (FT-IR)

The functional composition of the poly (ethylene glycol)-poly (propylene glycol)-poly (ethylene glycol) sol-gel sorbent coating is highlighted by FT-IR spectra, which also show how well they were integrated into the final obtained product. **Figures 2(a)** and **2(b)** in supplementary material depict the FT-IR spectra of poly (ethylene glycol)-poly (propylene glycol)-poly (ethylene glycol) (PEG-PPG-PEG) polymer and methyltrimethoxysilane (MTMS), respectively. **Figure 2(c)** illustrates the FT-IR spectrum of the PEG-PPG-PEG coated sol-gel FPSE membrane. As shown by the FT-IR spectra of methyltrimethoxysilane **(Figure 2(b))**, the bands at 1264 cm^{-1} and 789 cm^{-1} can be attributed to the vibration of the CH_3 functional group existing in the sol-gel precursor. The absorption band at 1102 cm^{-1} corresponds to the C–O stretching vibration of Si–O–CH_3, while the absorption bands at 2886 cm^{-1} and 1428

294 cm^{-1} correspond to the C–H stretching and bending vibrations of Si-O-CH_3, respectively [28].
295 The main bands in the poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol)
296 spectra are –C–H stretching at 2866 cm^{-1}, -C–H bending at 1455 cm^{-1}, and -C–O–C stretching
297 at 1095 cm^{-1} [29]. The presence of several bands in the FT-IR spectra of sol–gel poly(ethylene
298 glycol)-poly (propylene glycol)-poly(ethylene glycol) such as bands at 2886 cm^{-1}, 1428 cm^{-1},
299 1270 cm^{-1}, 1102 cm^{-1}, and 767 cm^{-1} (**Figure 2**) are also presented in the spectra of
300 methyltrimethoxysilane or in the FT-IR spectra of poly(propylene glycol)-poly(ethylene
301 glycol)-poly(propylene glycol), strongly signifying the successful embedding of precursor and
302 organic polymer within the sol-gel absorbent poly(propylene glycol)-poly(ethylene glycol)-
303 poly(propylene glycol).



304

305 **Figure 2.** FT-IR spectra of (a) pristine PEG-PPG-PEG polymer; (b) methyl trimethoxysilane
306 and (c) sol-gel PEG-PPG-PEG coated FPSE membrane

307

3.3 Optimization of FPSE conditions

Different experimental variables were used to test and iteratively improve the developed method's precision and recovery. Samples were prepared using FPSE in the procedure, and then HPLC-DAD was used to analyze the results.

3.3.1 Effect of pH on FPSE

The pH of the sample solutions is one of the most important parameters in the FPSE process, it can affect the molecular structure of all molecules in solution. Because of this, it is among the first parameters to be optimized. The optimal extraction efficiency is achieved when the target analytes, in their neutral state, interact with the extraction sorbent, which is also neutral. This maximizes the interactions between them, resulting in the highest possible extraction efficiency. By adding phosphate BR buffer solutions and adjusting the pH of the model solutions from 2 to 10, the fabric phase sorptive extraction technique (FPSE) was used to determine the pH that was most appropriate. According to the findings depicted in **Figure 3.** The optimal interaction between the analyte and the fabric phase was discovered to be pH 7 based on the data obtained. From the **Table 1**, it was determined that the pKa value of the chloramphenicol molecule was 9.5. This value explains why basic regions have better signals. Consequently, pH 7 BR buffer was used to carry out the remaining steps of the experimental studies.

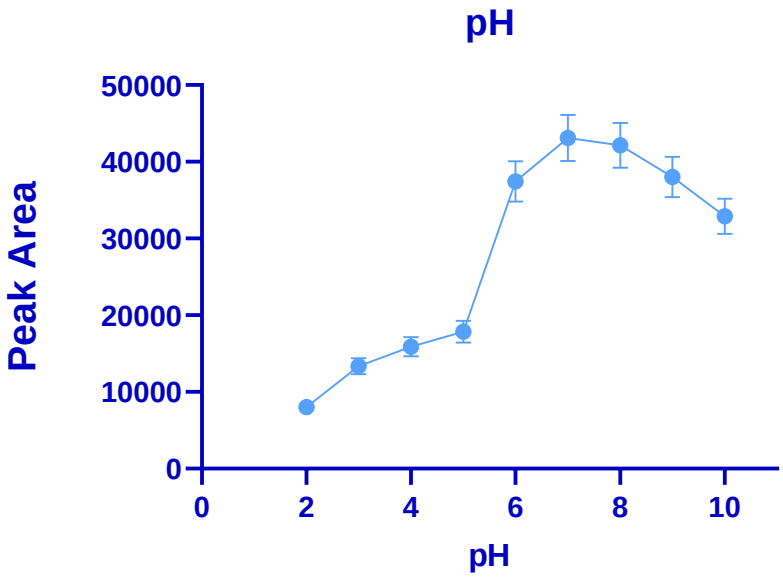


Figure 3. The effect of pH on the analytical signal (N:3)

3.3.2 Selection of desorption solvent and optimization of its volume

A strong elution solvent is anticipated to be necessary for full desorption of target molecules from the FPSE membrane surface. The solvent needs to work with the mobile phase system and HPLC machine. In this case, a series of numerous solvents including ethanol, methanol, acetonitrile, water, acetone, 2-propanol, *n*-hexane, acetonitrile: methanol, 50% methanol, and pH 5 buffer as mobile phase components were utilized to demonstrate the greatest signals for effective desorption conditions. Each solvent was added directly with 1 mL of volume to the FPSE membrane, which was then vortexed for 60s to eliminate CP. As can be seen in **Figure 4**, the optimal signals were produced with methanol for the CP. Regarding the desorption process, the use of methanol and acetonitrile allow to recover the highest amount of CP. Following the desorption results, and considering that simple alcohols (methanol, ethanol) or alkanes (heptane, hexane) are environmentally preferable solvents, whereas the use of dioxane, acetonitrile, acids, formaldehyde, and tetrahydrofuran is not recommendable from an environmental perspective, in the proposed FPSE procedure was selected methanol as desorption solvent. No carry over was observed because each sample treatment was performed on a new FPSE membrane.

Then, the volume of methanol was improved using model solutions. Because the volume of solvent directly influences the enrichment factor, the desorption solvent volume is a crucial component of the desorption procedure. The volume of the solvent should be as low as possible to attain a high enrichment factor. However, the desorption efficiency will be reduced if there is insufficient solvent since there will be inadequate contact between the solvent and the FPSE membrane. This step is crucial for the whole technique because, as expected, the enrichment factor declines as the solvent volume rises. Furthermore, it should be remembered that filtration of quantities less than 100 µL is difficult. According to this, volume optimization in the 100–1500 µL range was carried out. 400 µL of methanol were sufficient for a quantitative extraction, as shown in **Figure 5**. As anticipated, the signals first increased with volume before decreasing once further owing to the diluting effect. As a result, the 400 µL of methanol utilized in following experiments.

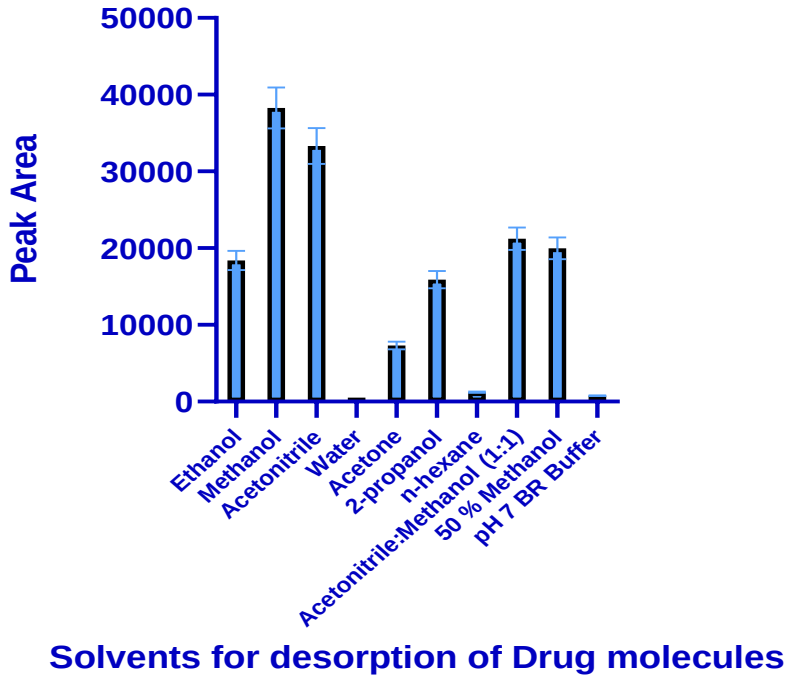


Figure 4. The effect of various solvents on desorption of Chloramphenicol molecules (N:3).

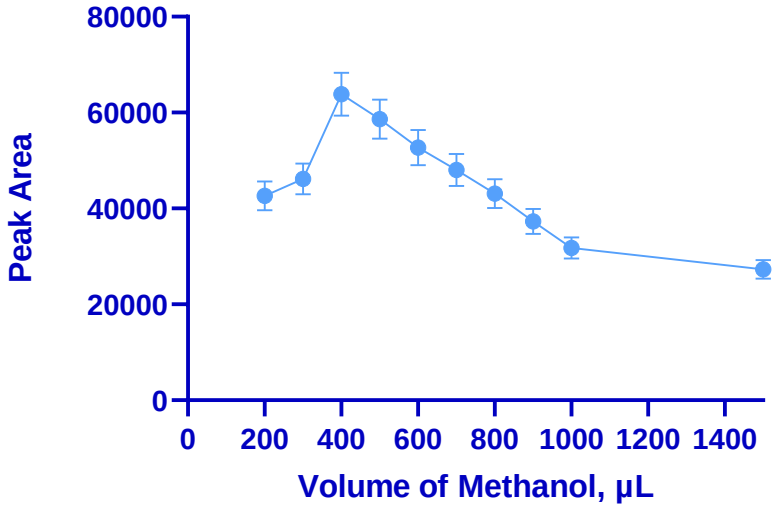


Figure 5. Effect of solvent volume on FPSE efficiency, (N:3)

3.3.3 Effect of interaction time between fabric phases and molecules

To achieve an efficient separation using the FPSE membrane in direct immersion extraction, a balance must be maintained over time between the aqueous sample substrate and the extraction sorbent. This equilibrium is influenced by the analyte partition coefficient between the extraction sorbent and the sample matrix. The time it takes for this equilibrium to form is influenced by a number of variables, such as temperature, viscosity of the sample matrix,

magnetic agitation or orbital agitation, etc. During the FPSE process, the target peak area rises until the extraction balance is attained. When the equilibrium is reached, further exposure to FPSE media does not lead to additional extraction. To maximize the extraction time, the orbital shaker's shaking period was investigated within the range of 0-90 min, while keeping all other parameters constant. The strongest signals for CP molecules were obtained after a 50 min agitation period, as depicted in **Figure 6**. The optimal signal was seen within 50 min, and there was no discernible rise in the signal for times longer than this period. This led to the agitation time for the next investigations being set for 50 min. Even while it may appear like a lengthy extraction process compared to other approaches, the ability to do many extractions at once shortens the overall analysis time for a set of samples.

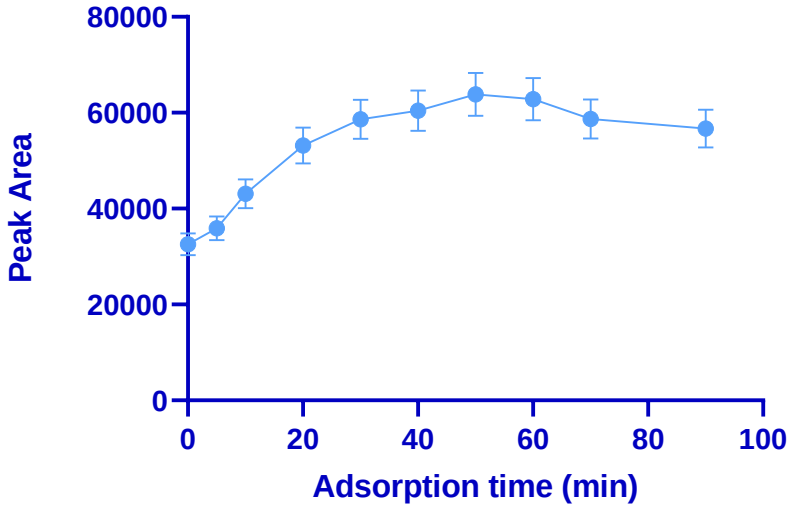


Figure 6. Effect of adsorption time on FPSE (N:3)

3.3.4 Effect of vortex time on the desorption of CP molecules

The desorption time of the vortex was carried out in the following optimization stage to determine the ideal time mandatory for the most efficient desorption of CP molecules. The time of the vortex process must be tuned while the other parameters were held constant in order to ensure the maximal transfer of molecules to solvent prior to HPLC analysis. As a result, model solutions containing a predetermined number of target molecules were created, and the timing of the vortex process was investigated between 0 and 90s. According to experimental data, a time of 20s was ideal for the quantitative desorption. As a result, the vortexing time for desorption in the following investigations will be 20s.

3.3.5 Analytical performance criteria

The developed FPSE-HPLC procedure was examined for a number of analytical performance parameters under ideal conditions, including the linear range, correlation coefficient, limit of detection (LOD), limit of quantification (LOQ), and reproducibility. The results are shown in **Table 1**. In a linear regression analysis, peak area against chloramphenicol molecular concentrations were employed. LOD was evaluated considering statistical 3 S_{blank}/m-criterion for ten replicate blank absorbance measurements, while LOQ was evaluated considering statistical 10 S_{blank}/m-criterion for 10 replicate blank absorbance measurements. The ratio of the volume of the initial solution (50 mL) to the volume of the eluent phase (400 µL) is known as the preconcentration factor (PF). To determine the enhancement factor (EF) of the proposed method, the ratio between the slope of the calibration curve obtained after pre-concentration and the slope of the calibration curve prior to pre-concentration was calculated. Additionally, the relative standard deviation (RSD%) was calculated using the proposed method for seven replicate analyses. These analyses included chloramphenicol molecules ranging from 25.0 to 1000.0 ng/mL.

Table 1: Analytical figure of merits of the developed method

Parameters	Before FPSE	After FPSE
Linear dynamic range	1.0-50.0 µg/mL	25.0-1000.0 ng/mL
Limit of detection (LOD) [a]	0.3 µg/mL	8.3 ng/mL
Limit of quantification (LOQ) [b]	0.9 µg/mL	25.0 ng/mL
RSD %	1.5	4.1
Calibration Sensitivity	5.045	585.22
Correlation coefficient (R^2)	0.9998	0.9942
Pre-concentration Factor [c]	-	125
Enhancement Factor [d]	-	85

[a] Based on statistical 3 S_{blank}/m-criterion for ten replicate blank absorbance measurements; [b] Based on statistical 10 S_{blank}/m-criterion for 10 replicate blank absorbance measurements; [c] Preconcentration factor is defined as the ratio of the initial solution volume (50 mL) to final volume of solution (400 µL); [d] Enhancement factor is defined as ratio of slope of calibration before and after FPSE.

3.3.6 Analysis of milk samples by using developed method

In this section, commercially available milk samples were used to evaluate the applicability of the herein proposed method. Samples were prepared as described in section 2.6. The FPSE method was developed by model solutions and milk samples submitted to developed method after a simple pretreatment procedure as explained in the related section. 2 mL of sample after

pre-treatment was transferred to FPSE system. The linearity and calibration were carried out by comparing peak area of target molecules in milk samples. Recovery and RSD% values were also calculated by using FPSE procedure obtained from milk samples.

In all the analyzed samples, CP concentration were found to be below the LOQ. For this reason, further analyses on fortified samples were performed also to evaluate and confirm the recovery. The recoveries obtained in each analyzed real sample were presented in **Table 2**. The obtained quantitative recoveries fell within the range of 93.0% and 106%, with a low relative standard deviation ranging between 2.7% and 5.7%. Chromatograms obtained from fortified real milk samples were also shown in **Figure 7**. As can be seen in **Figure 7**, peak of CP can be obvious after the proposed FPSE method without any interference in spiked milk samples.

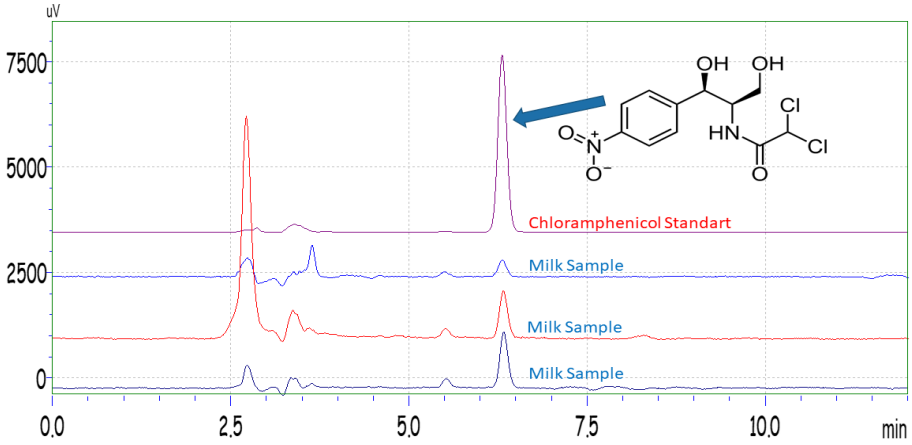


Figure 7. Chromatogram obtained from spiked milk samples

Table 2: Results for Chloramphenicol investigations and recovery tests in milk samples

Sample	Added ng/mL	Found[a] ng/mL	RSD %	Recovery %
Milk 1	0	<LOD	-	-
	250	242.5±12.5	5.2	97.0
	500	498.5±28.4	5.7	99.7
Milk 2	0	<LOD	-	-
	250	232.5±10.9	4.7	93.0
	500	487.5±21.4	4.3	99.7
Milk 3	0	<LOD	-	-
	250	265.3±12.4	4.7	106
	500	487.5±19.4	4.0	97.5
Milk 4	0	<LOD	-	-
	250	238.6±12.4	5.2	95.4
	500	496.5±13.2	2.7	99.3

[a]Mean ± standard deviation.

3.3.7 Comparison of analytical merits

The detection of CP antibiotic was compared with other reported methods using the newly developed and validated FPSE-HPLC-DAD method. A comparison list of several of these techniques is shown in **Table 3**. Component extraction in these previous investigations was carried out using traditional extraction methods including SPE and MSPE. The main benefit of the suggested method is that it provides a methodology that is simple to use for identifying antibiotic compounds in milk samples utilizing a traditional HPLC-DAD and FPSE. As previously mentioned, only a small volume of organic solvent is needed for quantitative elution with FPSE.

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

PRECONCENTRATION METHOD	METHOD	RECOVERY (%)	LOD	LOQ	RSD (%)	LINEAR RANGE	REF.
Solid-Phase Extraction	HPLC	90.3±3.6	0.45 µg/mL	1.52 µg/mL	1.66	2 – 10 µg/mL	[30]
Ionic Liquid-Anionic Surfactant Based Aqueous Two-Phase Extraction	HPLC	85.5 – 111	4.2 µg/kg	13.8 µg/kg	6.9	20.4 – 305.4 µg/kg	[31]
Magnetic Solid Phase Extraction	HPLC-PDA	94.6 – 105	3.02 ng/mL	9.63 ng/mL	4	10 – 600 ng/mL	[12]
Solid-Phase Extraction	HPLC-DAD	83.3 – 112	21.4 ng/mL	64.9 ng/mL	3.5-16.2	50 – 500 ng/mL	[6]
Fabric Phase Sorptive Extraction	HPLC–DAD	64.4 – 81.4	–	–	7.6-14	20 – 5000 µg/kg	[22]
Molecularly Imprinted Polymer Mixed With Solid Phase Extraction	HPLC	72.9 – 83.6	10 µg/kg	–	4.37	10 – 1000 µg/kg	[32]
Solid-Phase Extraction	HPLC-DAD	–	20 ng/mL	60 ng/mL	2.6	60 – 500 ng/mL	[33]
Magnetic Solid-Phase Extraction	SPE- HPLC-UV	85.5 – 105	10 mg	–	8.9	7 – 1000 µg/L	[34]
Solid-Phase Extraction	PT–SPE-HPLC–UV	92.7 – 99.8	0.01 µg/mL	0.03 µg/mL	<3.5	100 – 50000 ng/mL	[35]
Fabric Phase Sorptive Extraction	HPLC-DAD	93 – 106	8.3 ng/mL	25 ng/mL	2.7 – 5.7	25 – 1000 ng/mL	This Work

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As clearly highlighted, even if this procedure is focused on a single analyte respect a previously published paper [22], it represents a valid alternative in order to increase the throughput. In fact, it allows a similar analytical performance but using a shorter HPLC isocratic elution.

4. Conclusion

In this study, the FPSE-HPLC-DAD method was optimized and validated for rapidly determining CP in milk. Using this extraction approach during the sample preparation stage brought about several benefits, including less sample modification, avoiding protein precipitation or other purification procedures, and high recovery rates. This allow also to reduce the possible errors in the final quantitative measurements, as highlighted by the good analytical performances in terms of precision and trueness.

Optimum extraction efficiency was achieved by investigating all significant factors that may influence the extraction efficiency. While just 400 µL of MeOH was used for the analyte back-extraction process, it is essential to emphasize that this small volume of organic solvent is good enough to elute CP from the FPSE membranes quantitatively. This point is particularly important in terms of Greens Sample Preparation and especially related to the organic solvent consumption. A significant benefit of the proposed process is the volume decrease in the elution phase. It has been established that the approach is appropriate for milk sample analysis in terms of linearity, selectivity, trueness, and precision.

Further advantage is related to the isocratic elution, which reduce the drawbacks related to the method transferability, allowing having a powerful tool for fast CP determination.

In conclusion, the results of this research may encourage researchers to employ FPSE in everyday applications, and this approach may be applied in pharmaceutical quality control labs.

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.

Authorship contribution statement

Amina Ben Ayed: Investigation, Writing – original draft, Halil İbrahim Ulusoy: Data curation, Supervision, Project administration, Funding acquisition Ümmügülsüm Polat: Formal analysis, Validation Abuzar Kabir: Synthesis and Characterization, Data curation, Validation.

Songül Ulusoy: Conceptualization, Marcello Locatelli: Writing – original draft, Hamadi Khemakhem: Writing – review & editing

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A Facile Fabric Phase Sorptive Extraction Method for Monitoring Chloramphenicol Residues in Milk Samples

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Abstract

Determination of pharmaceutical elements and pharmacologically active molecules in the biological matrices is crucial in various fields of clinical and pharmaceutical chemistry, e.g., in pharmacokinetic studies, developing new drugs, or therapeutic drug monitoring. Chloramphenicol (CP) is used for treating bacterial infections, and it's one of the first antibiotics synthetically manufactured on a large scale. Fabric phase sorptive extraction (FPSE) was used to determine Chloramphenicol antibiotic residues in milk samples by means of validated HPLC-DAD instrumentation. Cellulose fabric phases modified with polyethylene glycol-block-polypropylene glycol-block-polyethylene glycol triblock copolymer was synthesized using sol-gel synthesis approach (Sol-gel PEG-PPG-PEG) and used for batch-type fabric phase extractions. Experimental variables of the FPSE method for antibiotic molecules were investigated and optimized systematically. The HPLC analysis of chloramphenicol was performed using a C18 column, isocratic elution of trifluoroacetic acid (0.1%), methanol, and acetonitrile (17:53:30) with a flow rate of 1.0 mL/min. The linear range for the proposed method for chloramphenicol ($r^2 > 0.9982$) was obtained in the range of 25.0–1000.0 ng/mL. The limit of detections (LOD) is 8.3 ng/mL, while RSDs% are below 4.1%. Finally, the developed method based on FPSE-HPLC-DAD was applied to milk samples to quantitatively determine antibiotic residues.

Keywords: Chloramphenicol; Fabric phase sorptive extraction; HPLC; Milk samples.

1. Introduction

The demand for food and other resources is rising as a result of the growing world population and shifting standards of life. Particularly, the need for healthy, safe food is rising, and the dairy industry has greatly benefited from this demand. Since ancient times, milk has been revered as nature's ideal diet and is a widely consumed commodity. It is a great source of nutrients and has the right proportions of proteins, lipids, carbs, vitamins, and minerals, which offers a number of advantages for development, immunity, and growth. With more than 6 billion customers worldwide, milk and milk products also contribute significantly to the growth of the food sector and economy. The presence of veterinary drug residues in milk, such as antibiotics, diminishes its nutritional value largely. Some scientists have found that the chemical residues in milk may contain medications used in veterinary therapies or from cleaning and other industrial activities in livestock facilities [1]. As a result, the quality of dairy products may be impacted by the presence of a wide range of organic chemical substances, including drugs, surfactants, and disinfectants. Due to antibiotic residues in foods of animal origin and water sources, humans are most impacted by poor cell membrane permeability, myalgia, skin rash, tendon rupture, hyperactivity, cancer, gastrointestinal and cardiovascular illnesses, etc.[2]. Antibiotics are heavily present in humans, animals, the environment, and food as a result of their widespread usage, low manufacturing costs, overuse, and abuse in the pharmaceutical and animal husbandry sectors of the economy. Because of this, certain microbes have become resistant to antibiotics [3]. Bacteria can proliferate and endure even in the presence of therapeutic dosages of antibiotics due to the adaptive genetic phenomena known as antibiotic resistance. Expressly, chloramphenicol (CP), also known as 2,2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl] acetamide, was discovered in 1947, in Streptomyces, Venezuela. The Parke-Davis team of researchers discovered and synthesized its molecular structure after two years in 1949 [4]. Chloramphenicol has been used to treat bacterial infections in people and animals. Additionally, CP is widely used in domestic poultry and animal feed production as a therapeutic and preventative agent against bacteria, for superficial eye infections, aquaculture, and beekeeping. This is owing to its low cost and great efficacy. CP misuse, on the other hand, has led to the entry of residues from animal products into the human body through the food chain and is the root of several chronic illnesses as cardiovascular failure, leukemia, and aplastic anemia. In **Table S1** (*Supplementary material*) were reported the main properties of CP. As a result, to maintain the safety of food items, the European Commission has set CP's minimum required performance limit (MRPL) at 0.3 µg/kg [5]. Thus, it is crucial to provide a quick and highly accurate approach for the monitoring and detection of CP in samples from various

clinical, environmental, and pharmacological sources. For the purpose of determining CP, a variety of analytical techniques and approaches have been created such as high-performance liquid chromatography (HPLC) [6], liquid chromatography-mass spectrometry (LC-MS) [7], gas chromatography-mass spectrometry (GC-MS) [8], gas chromatography (GC) [10], chemiluminescence [10] and capillary electrophoresis (CE) [11]. Due to the presence of these antibiotics in trace amounts in complex food matrices and the disruptive effects of matrix components, pre-separation and pre-concentration procedures are now required before these antibiotics are analyzed [12]. For the isolation and extraction of chloramphenicol, several sample classical techniques have been proposed such as the using of the salt-assisted liquid-liquid microextraction [13], magnetic solid phase extraction (MSPE) [12], solid-phase microextraction (SPME) [14], dual solid-phase microextraction [15], solid-phase extraction (SPE) [16], liquid-liquid extraction (LLE) [17]. Following the requirements of green analytical chemistry, modern analytical chemistry is now moving toward miniaturizing sample preparation and utilizing small quantities of organic solvent (or replace it). Additionally, several procedures require solvent evaporation and/or protein precipitation before the extraction, bringing to a significant loss of analytes. It is crucial to create sample preparation method that is simple, quick, efficient, and affordable, has a high analyte retention capacity, and permits regeneration for reuse of the same sorbent. For the above reasons, various novel extraction procedures that need less sample preparation and little sample volume have been developed recently.

In 2014, Kabir and Furton [18] created the fabric-phase sorptive extraction (FPSE), a modern sample preparation method, a cutting-edge sample preparation technique, combines the extraction mechanism of SPE and SPME into a single sample preparation platform. The FPSE provides a number of benefits such as it does not require sample pre-treatment procedures, such as filtration or centrifugation (or any other kind of pre-treatment procedure). In FPSE, a natural or synthetic fabric substrate is chemically treated to generate an ultra-thin coating of a hybrid sol-gel and organic-inorganic sorbent. A thin sorbent layer is formed during the chemically controlled sorbent coating process and then chemically bonds to the substrate surface. High porosity and easy permeability characterize the sol-gel sorbent-coated FPSE membrane. The use of FPSE has been reported earlier in numerous works for the extraction of developing pollutants, such as Venlafaxine [19], penicillin antibiotics [20], sulfonamides [21], amphenicols [22] and other compounds from raw or unprocessed milk with the least amount of sample preparation. Sensitive and selective findings for all types of samples may be produced using this approach in conjunction with a reliable separation and detection instrument.

The scope of the present research is to design and optimize for first time, a new analytical approach using sol–gel poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol)-modified cellulose fabric phase sorptive extraction (FPSE) membrane was exploited for the analysis of selected chloramphenicol antibiotic (CP) in milk samples prior to HPLC-DAD technique. Significant parameters such as the extraction time, sample volume and pH and elution solvent that influence the extraction effectiveness were carefully investigated. The adsorbent demonstrated good material performance, indicating that it might be exploited as a potential material in the development of FPSE technique with high analytical performance and it has effectively applied to the determination of the selected drugs in milk samples.

2. Materials and method

2.1 Chemicals and materials

The FPSE membranes used in this work were fabricated at the Department of Chemistry and Biochemistry, located in Florida International University, Miami, Florida, USA. In order to achieve the greatest quality possible, all chemicals, reagents, organic polymers, solvents and sol-gel precursor employed in the project were sourced from reputable commercial suppliers and ensured the highest quality available. Specifically, Methyltrimethoxysilane (MTMOS), trifluoroacetic acid (TFA), acetone, poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) polymer, and dichloromethane were obtained from Sigma-Aldrich, based in St. Louis, MO, USA. Additionally, muslin cotton fabric consisting of 100% cellulose was purchased from Jo-Ann Fabric in Miami, FL, USA. In the present project, all compounds employed were of high purity, with a minimum purity level of 99.5%. Deionized water with a resistivity of 18.2 MΩ cm, obtained from a MES Minipure Dest Up water purification system located in Ankara, Turkey, was used in all experimentations. For HPLC-DAD analysis, acetonitrile (ACN), methanol (MeOH), and trifluoroacetic acid (TFA) of HPLC-grade were utilized, sourced from Merck in Darmstadt, Germany. A stock solution of chloramphenicol at a concentration of 100 µg/mL (purchased from Sigma-Aldrich) was prepared using analytically pure methanol from Sigma Aldrich in St. Louis, MO, USA. Milk samples were obtained from local food stores in Sivas, Turkey, and various types of analyzed milk were stored refrigerated at a temperature of +4°C.

2.2 Instrumentation

An Eppendorf Centrifuge 5415 R (Eppendorf North America Inc., Hauppauge, NY, USA) was used to eliminate unwanted and interfering microparticles from the solutions prior to sol-

gel coating on the fabric substrate used to create FPSE membrane. For obtaining scanning electron microscope (SEM) images, a Philips XL30 Scanning Electron Microscope equipped with an EDAX detector was employed in this study. The SEM allowed for detailed visualization and analysis of the samples.

A Hettich Centrifuge (Universal 320, Hettich Lab Technology) was used to centrifugate various solutions in order to produce particle-free solutions. The full blending of several solutions was accomplished using a Fisher Scientific Digital shaker (Fisher brand) and a vortex mixer (Velp Scientifica F20220176 ZX3).

The pH readings were determined using a glass-calomel electrode pH meter (Mettler Toledo, Columbus, Ohio, OH, USA). For sample preparation, an ultrasonic water bath (made by Kudos, China) was employed. Prior to analysis, all chromatographic system solvents were degassed for 10 min in an ultrasonic bath (JP Selecta, Barcelona, Spain) and filtered through a 0.45 µm PTFE membrane filter (HNWP, Millipore) using a vacuum pump (Buchi, Switzerland).

2.3 Chromatographic analysis

The chromatographic setup used was equipped with a PDA detector SPD-M20A, an auto sampler SIL-20Ac, a thermostatic oven CTO-10 AS, and a pump type LC20-AD from Shimadzu, Tokyo, Japan (Shimadzu). LC solution software was used to transmit the obtained data to the computer (Shimadzu).

The HPLC analysis of CP was performed using a C18 column (Luna Omega C18, 250 mm x 4.6 mm, 5.0 µm) in isocratic elution mode using trifluoroacetic acid (0.1%), methanol, and acetonitrile (17:53:30, *v:v:v*) with a flow rate of 1.0 mL/min. The column was maintained at 40°C, while for quantitative analyses the detector wavelength was set at 276 nm. The injection volume was 10 µL. Prior to analysis, samples and mobile phases were ultrasonically degassed for 10 min and filtered using a membrane filter (0.45 µm). By comparing retention times and UV/Vis spectra of standards, each peak in the chromatograms was recognized. Analytical results were quantified by peak area at the respective analyte maximum wavelengths. Additional information about the HPLC configuration and setting were reported in **section S2** (*supplementary material*).

2.4 Preparation of Sol-gel PEG-PPG-PEG coated FPSE membrane

Commercial 100% cellulose cotton fabric was selected as the substrate for FPSE membrane. To remove any potential residual chemicals, present in the commercial source of the fabric and

to maximize the surface hydroxyl groups of the cellulose fabric, the fabric substrate was treated with 1M NaOH solution for 1h followed by rinsing with deionized water several times and subsequently treated with 0.1M HCl to neutralize any remnant of NaOH potentially present in the fabric substrate. The detail procedure of the fabric substrate treatment is presented elsewhere [23,24].

In order to ensure maximum extraction efficiency for the target analyte, a medium polarity sorbent, sol-gel PEG-PPG-PEG was designed and synthesized. The sol solution for creating the thin layer coating on the substrate surface was composed of an organically modified inorganic precursor, methyl trimethoxysilane (MTMS), an organic polymer, PEG-PPG-PEG, a solvent system (50:50, *v:v* methylene chloride: acetone), an acid catalyst (trifluoroacetic acid, TFA), and water. The molar ratio between the sol-gel precursor, organic/inorganic polymer, acetone, methylene chloride, TFA and water was optimized. The optimum molar ratio of the building blocks was maintained at: 1: 0.1: 3.26: 3.74: 1.25: 3 The detail process for preparing the sol solution and the sol-gel sorbent coating process via immersion-coating technology are presented elsewhere [24–26].

Briefly, building blocks of the individual sol solution were added sequentially into 50 mL high-density polyethylene centrifuge tube followed by vortexing for 3 min. Finally, the sol solution was centrifuged at 14000 rpm to remove any particulate matter suspended in the solution. The supernatant of the sol solution was then transferred in 50 mL amber glass reaction vessel and a pre-treated fabric (30 cm x 20 cm) was gently inserted in the sol solution to initiate the immersion coating process. The sol-gel sorbent coating continued for 6h at room temperature. Subsequently, the fabric substrate was removed from the sol-gel reaction vessel and air-dried for 1h. The sol-gel sorbent-coated FPSE membrane was then subjected to thermal conditioning in an inert environment at 50°C for 24h under continuous helium gas flow. The FPSE membrane was then rinsed with 50:50 (*v:v*) methylene chloride: methanol, air dried for 1h and thermally conditioned at 50°C for 8h. The FPSE membrane was cut into 1.0 cm x 1.0 cm units and stored in airtight container until their use in FPSE experiments.

2.5 FPSE procedure

The FPSE membrane was cleaned by immersing it in 2 mL of acetonitrile: methanol (50:50, *v:v*) for 5 min. No vortexing, shaking, or stirring was needed since the organic solvent combination may quickly penetrate through the porous sol-gel coating and permeable substrate matrix. The remaining organic solvents were washed from the FPSE membrane using 2 mL of deionized water. 2.0 mL of pH 7 buffer and 1 cm^2 of the fabric phase were added to 20 mL of

sample solution containing chloramphenicol in the range of 25-1000 ng/mL, and the final volume was raised to 50 mL with deionized water. After that, an orbital shaker was used to increase interactions between fabric phase and drug molecules for 50 min at 50 rpm. Each FPSE membrane was taken out and then, using 400 µL of methanol, the chloramphenicol was back-extracted. Vortex provided assistance with back-extraction for 20s. Finally, 0.45 µm membrane filters were used to filter the back-extracted solutions before the HPLC analysis.

2.6 Preparation of milk samples

To evaluate the effectiveness of the developed technique, real milk samples were employed. The milk samples underwent a straightforward procedure with minor modifications, as outlined in a previously published article [27]. In this process, 5 mL of homogenized milk was dropped into a 50 mL centrifuge tube. Subsequently, it was mixed vigorously using a vortex with 0.5 mL of 0.50% (*w:w*) ascorbic acid and 2 mL of methanol. The mixture was then shaken for 1 min using a vortex mixer. Following this, the samples were subjected to centrifugation at 3000 rpm for 5 min. The resulting supernatant phase was filtered through a 0.45 µm membrane filter. Finally, 2 mL of the filtered solution was utilized for fabric phase sorptive extraction using the developed method.

3. Results and discussion

3.1 Mechanism of extraction on the FPSE membrane

Major analytical challenges appear when the target analyte is relatively polar and dispersed in a complex sample matrix. Milk contains numerous matrix interferents including proteins, fats, salts, and many others. Sol-gel derived sorbent in FPSE, unlike sorbents in classical extraction and microextraction techniques, extracts analyte from the sample matrix by exerting different intermolecular interaction including London dispersion, hydrophobic/hydrophilic surface properties, dipole-dipole interactions, and hydrogen bonding towards the target analytes. The primary extraction mechanism of sol-gel PEG-PPG-PEG sorbent-coated FPSE membrane is governed by the adsorption of target analytes onto the surface of the sponge-like porous sol-gel sorbent via intermolecular interactions between the sorbent and the analytes. The sponge-like porous morphology of the sol-gel sorbent allows rapid permeation of the aqueous sample carrying the target analytes for interacting with the sorbent, resulting in fast extraction kinetic and short overall sample preparation time. Chloramphenicol is a medium polar analyte with a log Kow value of 1.14. A rational polymer selection for this analyte leads to medium polar polymer PEG-PPG-PEG. Due to the polymer segment connected as blocks, this unique

polymer exerts affinity towards polar and medium polar analytes very efficiently via different intermolecular interactions, resulting in high extraction efficiency for a wide range of analytes. As expected, the sol-gel PEG-PPG-PEG sorbent-coated FPSE membrane demonstrated very high affinity towards chloramphenicol as manifested by low limit of detection compared to other published analytical methods developed for chloramphenicol.

3.2 Characterization of sol–gel PEG-PPG-PEG coated FPSE membrane

3.2.1 Scanning electron microscopy

Scanning electron microscopy (SEM) was used to examine the FPSE membrane's surface morphology. FPSE media exploit a number of merits offered by sol-gel coating technology, an extremely regulated surface coating method that ensures unrivaled coating homogeneity and chemical bonding between the sol-gel sorbent and the substrate. FPSE links solid phase extraction (characterized by exhaustive principle) and the extraction principles of solid phase microextraction (characterized by equilibrium extraction) by its unique design. In order to apply the concept of exhaustive extraction, the FPSE membrane must be permeable. As shown in **Figure 1 (a)**, cotton fabrics (100% cellulose) are designed to have good ventilation. **Figure 1 (b)** shows the surface morphology of the uncoated cellulose fabric at a magnification of 500x to show the individual microfibrils, which serve as the foundation for woven fabric, have a smooth, seemingly polished surface. SEM image of the sol-gel PEG-PPG-PEG coated FPSE membrane is shown in **Figure 1(c)** at 100x magnification. As can be seen, the through-holes in the FPSE membrane are still present, even after sol-gel coating of the sorbent. The benefit and ease of the sol-gel sorbent coating technique is demonstrated by a consistent coating surrounding each microfibril of the cellulose fabric as seen in **Figure 1 (d)** at 500x magnifications.

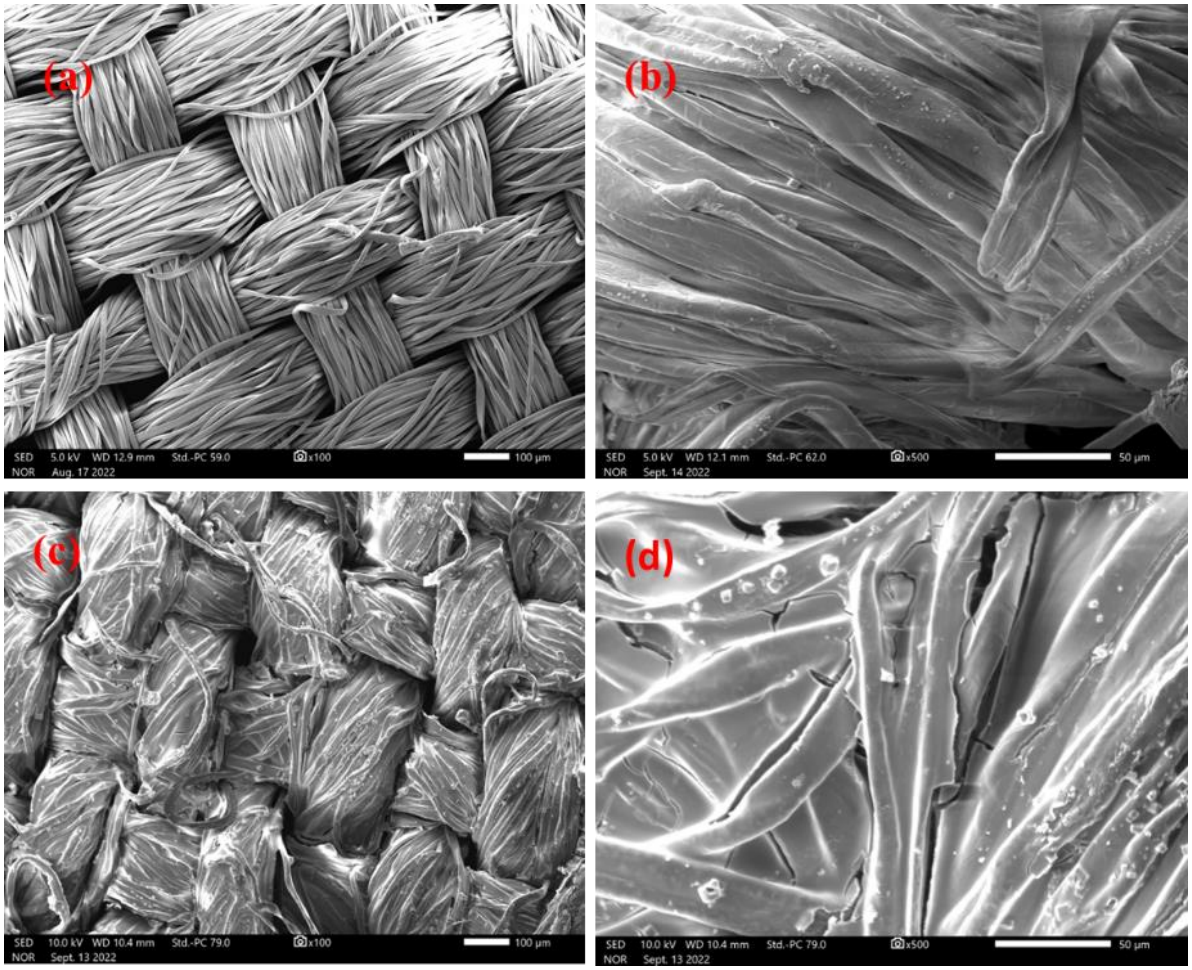
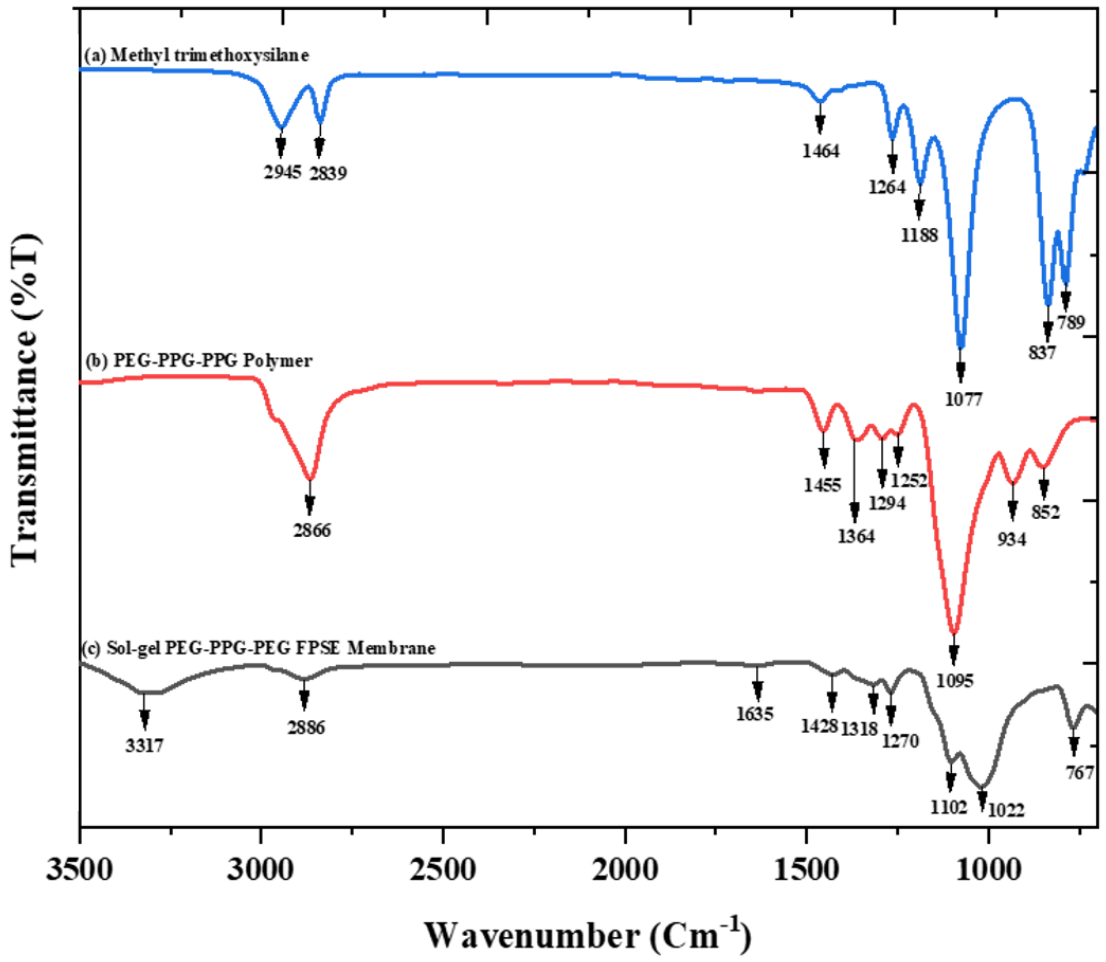


Figure 1. SEM images of (a) uncoated cellulose cotton fabric surface at 100x magnifications; (b) uncoated cellulose fabric surface at 500x magnifications; (c) sol-gel PEG-PPG-PEG coated cellulose fabric surface at 100x magnifications; (d) sol-gel PEG-PPG-PEG coated cellulose fabric surface at 500x magnifications.

3.2.2 Fourier Transform Infrared Spectroscopy (FT-IR)

The functional composition of the poly (ethylene glycol)-poly (propylene glycol)-poly (ethylene glycol) sol-gel sorbent coating is highlighted by FT-IR spectra, which also show how well they were integrated into the final obtained product. **Figures 2(a)** and **2(b)** in supplementary material depict the FT-IR spectra of poly (ethylene glycol)-poly (propylene glycol)-poly (ethylene glycol) (PEG-PPG-PEG) polymer and methyltrimethoxysilane (MTMS), respectively. **Figure 2(c)** illustrates the FT-IR spectrum of the PEG-PPG-PEG coated sol-gel FPSE membrane. As shown by the FT-IR spectra of methyltrimethoxysilane **(Figure 2(b))**, the bands at 1264 cm^{-1} and 789 cm^{-1} can be attributed to the vibration of the CH_3 functional group existing in the sol-gel precursor. The absorption band at 1102 cm^{-1} corresponds to the C–O stretching vibration of Si–O–CH_3, while the absorption bands at 2886 cm^{-1} and 1428

294 cm^{-1} correspond to the C–H stretching and bending vibrations of Si-O-CH_3, respectively [28].
295 The main bands in the poly(ethylene glycol)-poly(propylene glycol)-poly(ethylene glycol)
296 spectra are –C–H stretching at 2866 cm^{-1}, -C–H bending at 1455 cm^{-1}, and -C–O–C stretching
297 at 1095 cm^{-1} [29]. The presence of several bands in the FT-IR spectra of sol–gel poly(ethylene
298 glycol)-poly (propylene glycol)-poly(ethylene glycol) such as bands at 2886 cm^{-1}, 1428 cm^{-1},
299 1270 cm^{-1}, 1102 cm^{-1}, and 767 cm^{-1} (**Figure 2**) are also presented in the spectra of
300 methyltrimethoxysilane or in the FT-IR spectra of poly(propylene glycol)-poly(ethylene
301 glycol)-poly(propylene glycol), strongly signifying the successful embedding of precursor and
302 organic polymer within the sol-gel absorbent poly(propylene glycol)-poly(ethylene glycol)-
303 poly(propylene glycol).



304

305 **Figure 2.** FT-IR spectra of (a) pristine PEG-PPG-PEG polymer; (b) methyl trimethoxysilane
306 and (c) sol-gel PEG-PPG-PEG coated FPSE membrane

307

3.3 Optimization of FPSE conditions

Different experimental variables were used to test and iteratively improve the developed method's precision and recovery. Samples were prepared using FPSE in the procedure, and then HPLC-DAD was used to analyze the results.

3.3.1 Effect of pH on FPSE

The pH of the sample solutions is one of the most important parameters in the FPSE process, it can affect the molecular structure of all molecules in solution. Because of this, it is among the first parameters to be optimized. The optimal extraction efficiency is achieved when the target analytes, in their neutral state, interact with the extraction sorbent, which is also neutral. This maximizes the interactions between them, resulting in the highest possible extraction efficiency. By adding phosphate BR buffer solutions and adjusting the pH of the model solutions from 2 to 10, the fabric phase sorptive extraction technique (FPSE) was used to determine the pH that was most appropriate. According to the findings depicted in **Figure 3.** The optimal interaction between the analyte and the fabric phase was discovered to be pH 7 based on the data obtained. From the **Table 1**, it was determined that the pKa value of the chloramphenicol molecule was 9.5. This value explains why basic regions have better signals. Consequently, pH 7 BR buffer was used to carry out the remaining steps of the experimental studies.

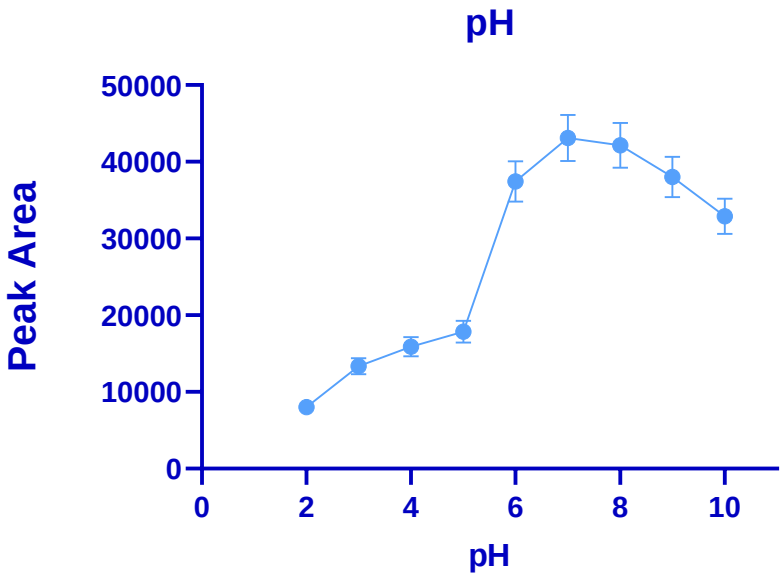


Figure 3. The effect of pH on the analytical signal (N:3)

3.3.2 Selection of desorption solvent and optimization of its volume

A strong elution solvent is anticipated to be necessary for full desorption of target molecules from the FPSE membrane surface. The solvent needs to work with the mobile phase system and HPLC machine. In this case, a series of numerous solvents including ethanol, methanol, acetonitrile, water, acetone, 2-propanol, *n*-hexane, acetonitrile: methanol, 50% methanol, and pH 5 buffer as mobile phase components were utilized to demonstrate the greatest signals for effective desorption conditions. Each solvent was added directly with 1 mL of volume to the FPSE membrane, which was then vortexed for 60s to eliminate CP. As can be seen in **Figure 4**, the optimal signals were produced with methanol for the CP. Regarding the desorption process, the use of methanol and acetonitrile allow to recover the highest amount of CP. Following the desorption results, and considering that simple alcohols (methanol, ethanol) or alkanes (heptane, hexane) are environmentally preferable solvents, whereas the use of dioxane, acetonitrile, acids, formaldehyde, and tetrahydrofuran is not recommendable from an environmental perspective, in the proposed FPSE procedure was selected methanol as desorption solvent. No carry over was observed because each sample treatment was performed on a new FPSE membrane.

Then, the volume of methanol was improved using model solutions. Because the volume of solvent directly influences the enrichment factor, the desorption solvent volume is a crucial component of the desorption procedure. The volume of the solvent should be as low as possible to attain a high enrichment factor. However, the desorption efficiency will be reduced if there is insufficient solvent since there will be inadequate contact between the solvent and the FPSE membrane. This step is crucial for the whole technique because, as expected, the enrichment factor declines as the solvent volume rises. Furthermore, it should be remembered that filtration of quantities less than 100 µL is difficult. According to this, volume optimization in the 100–1500 µL range was carried out. 400 µL of methanol were sufficient for a quantitative extraction, as shown in **Figure 5**. As anticipated, the signals first increased with volume before decreasing once further owing to the diluting effect. As a result, the 400 µL of methanol utilized in following experiments.

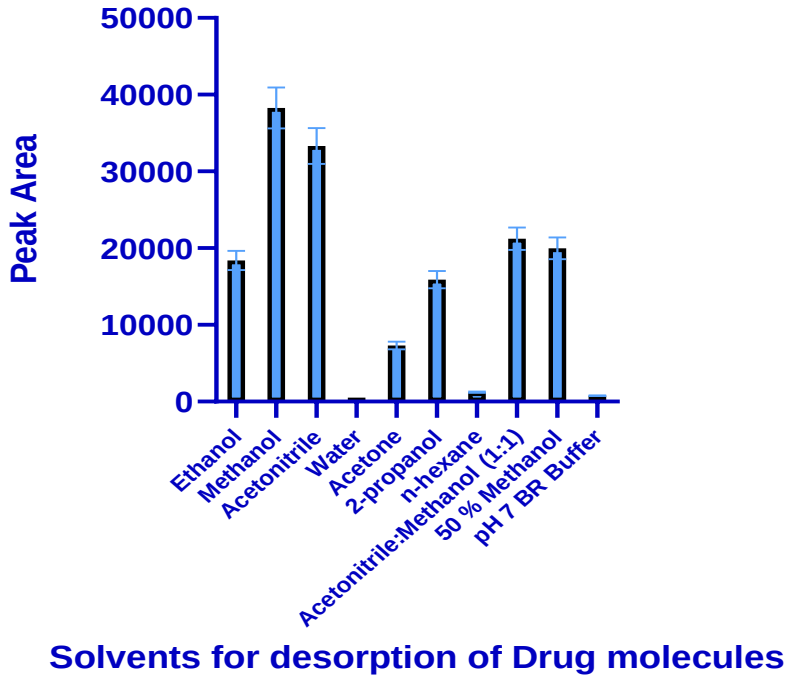


Figure 4. The effect of various solvents on desorption of Chloramphenicol molecules (N:3).

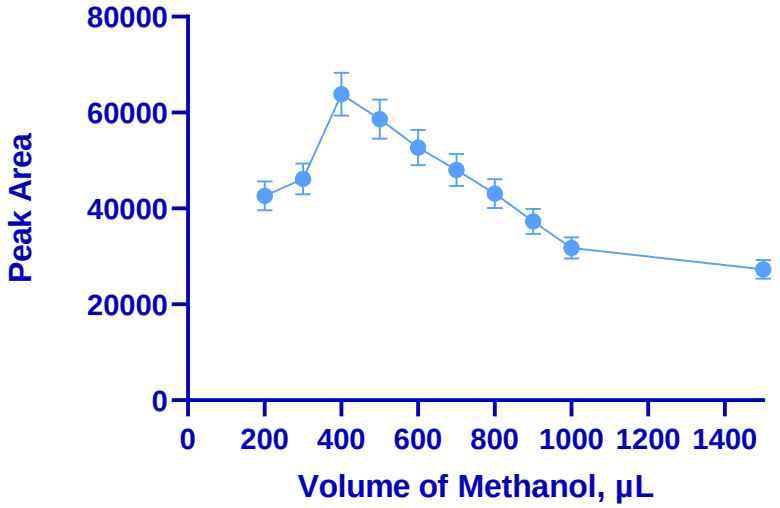


Figure 5. Effect of solvent volume on FPSE efficiency, (N:3)

3.3.3 Effect of interaction time between fabric phases and molecules

To achieve an efficient separation using the FPSE membrane in direct immersion extraction, a balance must be maintained over time between the aqueous sample substrate and the extraction sorbent. This equilibrium is influenced by the analyte partition coefficient between the extraction sorbent and the sample matrix. The time it takes for this equilibrium to form is influenced by a number of variables, such as temperature, viscosity of the sample matrix,

magnetic agitation or orbital agitation, etc. During the FPSE process, the target peak area rises until the extraction balance is attained. When the equilibrium is reached, further exposure to FPSE media does not lead to additional extraction. To maximize the extraction time, the orbital shaker's shaking period was investigated within the range of 0-90 min, while keeping all other parameters constant. The strongest signals for CP molecules were obtained after a 50 min agitation period, as depicted in **Figure 6**. The optimal signal was seen within 50 min, and there was no discernible rise in the signal for times longer than this period. This led to the agitation time for the next investigations being set for 50 min. Even while it may appear like a lengthy extraction process compared to other approaches, the ability to do many extractions at once shortens the overall analysis time for a set of samples.

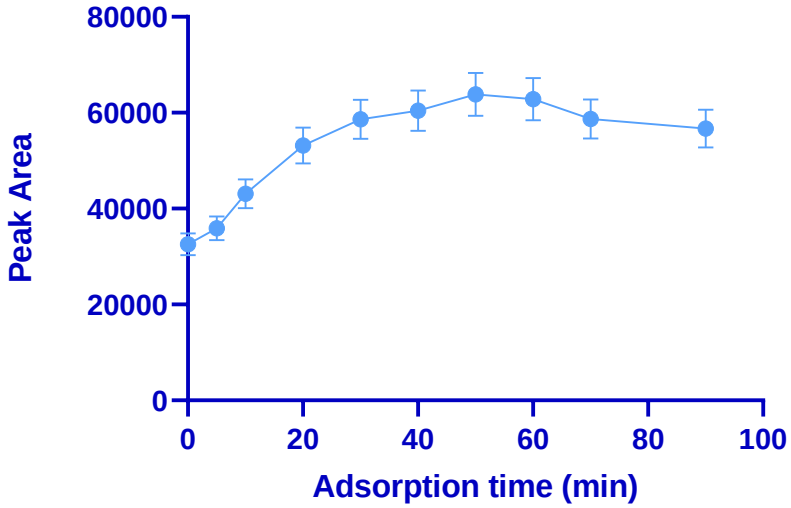


Figure 6. Effect of adsorption time on FPSE (N:3)

3.3.4 Effect of vortex time on the desorption of CP molecules

The desorption time of the vortex was carried out in the following optimization stage to determine the ideal time mandatory for the most efficient desorption of CP molecules. The time of the vortex process must be tuned while the other parameters were held constant in order to ensure the maximal transfer of molecules to solvent prior to HPLC analysis. As a result, model solutions containing a predetermined number of target molecules were created, and the timing of the vortex process was investigated between 0 and 90s. According to experimental data, a time of 20s was ideal for the quantitative desorption. As a result, the vortexing time for desorption in the following investigations will be 20s.

3.3.5 Analytical performance criteria

The developed FPSE-HPLC procedure was examined for a number of analytical performance parameters under ideal conditions, including the linear range, correlation coefficient, limit of detection (LOD), limit of quantification (LOQ), and reproducibility. The results are shown in **Table 1**. In a linear regression analysis, peak area against chloramphenicol molecular concentrations were employed. LOD was evaluated considering statistical 3 S_{blank}/m-criterion for ten replicate blank absorbance measurements, while LOQ was evaluated considering statistical 10 S_{blank}/m-criterion for 10 replicate blank absorbance measurements. The ratio of the volume of the initial solution (50 mL) to the volume of the eluent phase (400 µL) is known as the preconcentration factor (PF). To determine the enhancement factor (EF) of the proposed method, the ratio between the slope of the calibration curve obtained after pre-concentration and the slope of the calibration curve prior to pre-concentration was calculated. Additionally, the relative standard deviation (RSD%) was calculated using the proposed method for seven replicate analyses. These analyses included chloramphenicol molecules ranging from 25.0 to 1000.0 ng/mL.

Table 1: Analytical figure of merits of the developed method

Parameters	Before FPSE	After FPSE
Linear dynamic range	1.0-50.0 µg/mL	25.0-1000.0 ng/mL
Limit of detection (LOD) [a]	0.3 µg/mL	8.3 ng/mL
Limit of quantification (LOQ) [b]	0.9 µg/mL	25.0 ng/mL
RSD %	1.5	4.1
Calibration Sensitivity	5.045	585.22
Correlation coefficient (R^2)	0.9998	0.9942
Pre-concentration Factor [c]	-	125
Enhancement Factor [d]	-	85

[a] Based on statistical 3 S_{blank}/m-criterion for ten replicate blank absorbance measurements; [b] Based on statistical 10 S_{blank}/m-criterion for 10 replicate blank absorbance measurements; [c] Preconcentration factor is defined as the ratio of the initial solution volume (50 mL) to final volume of solution (400 µL); [d] Enhancement factor is defined as ratio of slope of calibration before and after FPSE.

3.3.6 Analysis of milk samples by using developed method

In this section, commercially available milk samples were used to evaluate the applicability of the herein proposed method. Samples were prepared as described in section 2.6. The FPSE method was developed by model solutions and milk samples submitted to developed method after a simple pretreatment procedure as explained in the related section. 2 mL of sample after

pre-treatment was transferred to FPSE system. The linearity and calibration were carried out by comparing peak area of target molecules in milk samples. Recovery and RSD% values were also calculated by using FPSE procedure obtained from milk samples.

In all the analyzed samples, CP concentration were found to be below the LOQ. For this reason, further analyses on fortified samples were performed also to evaluate and confirm the recovery. The recoveries obtained in each analyzed real sample were presented in **Table 2**. The obtained quantitative recoveries fell within the range of 93.0% and 106%, with a low relative standard deviation ranging between 2.7% and 5.7%. Chromatograms obtained from fortified real milk samples were also shown in **Figure 7**. As can be seen in **Figure 7**, peak of CP can be obvious after the proposed FPSE method without any interference in spiked milk samples.

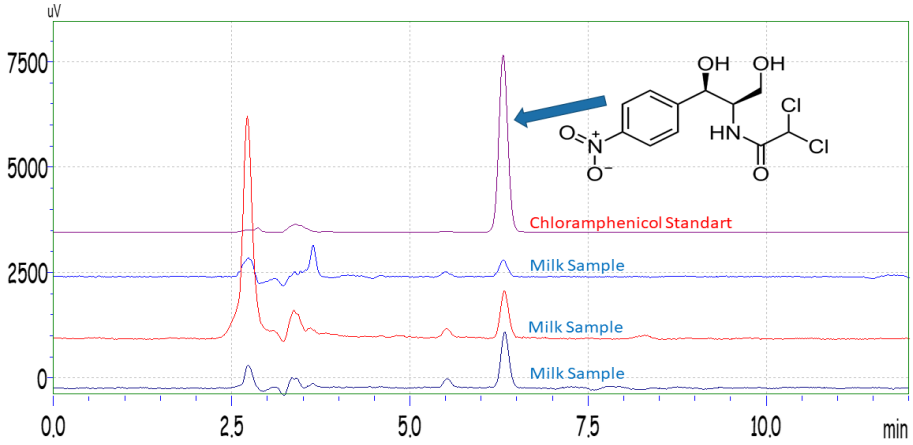


Figure 7. Chromatogram obtained from spiked milk samples

Table 2: Results for Chloramphenicol investigations and recovery tests in milk samples

Sample	Added ng/mL	Found[a] ng/mL	RSD %	Recovery %
Milk 1	0	<LOD	-	-
	250	242.5±12.5	5.2	97.0
	500	498.5±28.4	5.7	99.7
Milk 2	0	<LOD	-	-
	250	232.5±10.9	4.7	93.0
	500	487.5±21.4	4.3	99.7
Milk 3	0	<LOD	-	-
	250	265.3±12.4	4.7	106
	500	487.5±19.4	4.0	97.5
Milk 4	0	<LOD	-	-
	250	238.6±12.4	5.2	95.4
	500	496.5±13.2	2.7	99.3

[a]Mean ± standard deviation.

3.3.7 Comparison of analytical merits

The detection of CP antibiotic was compared with other reported methods using the newly developed and validated FPSE-HPLC-DAD method. A comparison list of several of these techniques is shown in **Table 3**. Component extraction in these previous investigations was carried out using traditional extraction methods including SPE and MSPE. The main benefit of the suggested method is that it provides a methodology that is simple to use for identifying antibiotic compounds in milk samples utilizing a traditional HPLC-DAD and FPSE. As previously mentioned, only a small volume of organic solvent is needed for quantitative elution with FPSE.

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

PRECONCENTRATION METHOD	METHOD	RECOVERY (%)	LOD	LOQ	RSD (%)	LINEAR RANGE	REF.
Solid-Phase Extraction	HPLC	90.3±3.6	0.45 µg/mL	1.52 µg/mL	1.66	2 – 10 µg/mL	[30]
Ionic Liquid-Anionic Surfactant Based Aqueous Two-Phase Extraction	HPLC	85.5 – 111	4.2 µg/kg	13.8 µg/kg	6.9	20.4 – 305.4 µg/kg	[31]
Magnetic Solid Phase Extraction	HPLC-PDA	94.6 – 105	3.02 ng/mL	9.63 ng/mL	4	10 – 600 ng/mL	[12]
Solid-Phase Extraction	HPLC-DAD	83.3 – 112	21.4 ng/mL	64.9 ng/mL	3.5-16.2	50 – 500 ng/mL	[6]
Fabric Phase Sorptive Extraction	HPLC–DAD	64.4 – 81.4	–	–	7.6-14	20 – 5000 µg/kg	[22]
Molecularly Imprinted Polymer Mixed With Solid Phase Extraction	HPLC	72.9 – 83.6	10 µg/kg	–	4.37	10 – 1000 µg/kg	[32]
Solid-Phase Extraction	HPLC-DAD	–	20 ng/mL	60 ng/mL	2.6	60 – 500 ng/mL	[33]
Magnetic Solid-Phase Extraction	SPE- HPLC-UV	85.5 – 105	10 mg	–	8.9	7 – 1000 µg/L	[34]
Solid-Phase Extraction	PT–SPE-HPLC–UV	92.7 – 99.8	0.01 µg/mL	0.03 µg/mL	<3.5	100 – 50000 ng/mL	[35]
Fabric Phase Sorptive Extraction	HPLC-DAD	93 – 106	8.3 ng/mL	25 ng/mL	2.7 – 5.7	25 – 1000 ng/mL	This Work

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As clearly highlighted, even if this procedure is focused on a single analyte respect a previously published paper [22], it represents a valid alternative in order to increase the throughput. In fact, it allows a similar analytical performance but using a shorter HPLC isocratic elution.

4. Conclusion

In this study, the FPSE-HPLC-DAD method was optimized and validated for rapidly determining CP in milk. Using this extraction approach during the sample preparation stage brought about several benefits, including less sample modification, avoiding protein precipitation or other purification procedures, and high recovery rates. This allow also to reduce the possible errors in the final quantitative measurements, as highlighted by the good analytical performances in terms of precision and trueness.

Optimum extraction efficiency was achieved by investigating all significant factors that may influence the extraction efficiency. While just 400 µL of MeOH was used for the analyte back-extraction process, it is essential to emphasize that this small volume of organic solvent is good enough to elute CP from the FPSE membranes quantitatively. This point is particularly important in terms of Greens Sample Preparation and especially related to the organic solvent consumption. A significant benefit of the proposed process is the volume decrease in the elution phase. It has been established that the approach is appropriate for milk sample analysis in terms of linearity, selectivity, trueness, and precision.

Further advantage is related to the isocratic elution, which reduce the drawbacks related to the method transferability, allowing having a powerful tool for fast CP determination.

In conclusion, the results of this research may encourage researchers to employ FPSE in everyday applications, and this approach may be applied in pharmaceutical quality control labs.

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.

Authorship contribution statement

Amina Ben Ayed: Investigation, Writing – original draft, Halil İbrahim Ulusoy: Data curation, Supervision, Project administration, Funding acquisition Ümmügülsüm Polat: Formal analysis, Validation Abuzar Kabir: Synthesis and Characterization, Data curation, Validation.

Songül Ulusoy: Conceptualization, Marcello Locatelli: Writing – original draft, Hamadi Khemakhem: Writing – review & editing

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Declaration of interests

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