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Fabric phase sorptive extraction combined with high performance liquid chromatography for the determination of favipiravir in human plasma and breast milk

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Abstract

A fast procedure obtained by the combination of fabric phase extraction (FPSE) with high performance liquid chromatography (HPLC) has been developed and validated for the quantification of favipiravir (FVP) in human plasma and breast milk. A sol-gel polycaprolactone-block-polydimethylsiloxane-block-polycaprolactone (sol-gel PCAP-PDMS-PCAP) coated on 100% cellose cotton fabric was selected as the most efficient membrane for FPSE in human plasma and breast milk samples. HPLC-UV analysis were performed using a RP C18 column under isocratic conditions. Under these optimezed settings, the overall chromatographic analysis time was limited to only 5 min. without encountering any observable matrix interferences.

Following the method validation procedure, the herein assay shows a linear calibration curve over the range of 0.2-50 µg/mL and 0.5-25 µg/mL for plasma and breast milk, respectively. The method sensitivities in terms of limit of detection (LOD) and limit of quantification (LOQ), validated in both the matrices, have been found to be 0.06 and 0.2 µg/mL for plasma and 0.15 and 0.5 µg/mL for milk, respectively. Intraday and interday precision and trueness, accordingly to the International Guidelines, were validated and were below 3.61% for both the matrices.

The herein method was further tested on real samples in order to highlight the applicability and the advantage for therapeutic drug monitoring (TDM) applications. To the best of our knowledge, this is the first validated FPSE-HPLC-UV method in human plasma and breast milk for TDM purposes applied on real samples. The validated method provides fast, simple, cost reduced, and sensitive assay for the direct quantification of favipiravir in real biological matrices, also appliyng a well-known rugged and cheap instrument configuration.

Keywords: HPLC, favipiravir, fabric phase sorptive extraction, plasma, breast milk, TDM

1. Introduction

Favipiravir (FVP, 6-fluoro-2-oxo-1H-pyrazine-3-carboxamide), is an antiviral drug used to treat cases of influenza that are resistant to the conventional treatments. Furthermore, after COVID-19 pandemic, according to the clinical trials it became a promising antiviral agent for corona virus [1]. It inhibits the enzyme required for RNA viral replication in human cells called RNA-polymerase and functions as a purine analogue incorporated instead of guanine and adenine [2], and was also used for prophylaxis and treatment of Ebola virus infections [3]. Due to the popularity and wide application in COVID-19 pandemic, its quantification in human plasma for therapeutic drug monitoring purposes (TDM), and the amount of elimination from breast milk gained high importance.

Due to the emergency use of FVP, it is allowed to use high doses up to 1600 mg/day to treat the diseases caused by COVID-19, but in the meantime the side effects have to be deeply investigated and carefully monitorited. There are no data available to recognize the effects in infants, and in this scenario, the exposure derived from the breast milk is a critical issue to obtain right and useful information.

In the literature, there are just few methods for plasma analysis, but there is no method available for the determination of FVP in other human biological fluids. Particularly, there are three methods for spiked plasma samples [4-6], two of them based on reversed phase-high performance liquid chromatography (RP-HPLC) with ultraviolet detection (UV) [4,5], and one is based on a spectrofluorimetric method [6]. In literature were reported two liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods for plasma analysis conducted on healthy volunteers that are feasible for bioequivalance studies [7,8], and other two methods related to the quantification of the active principle (FVP) in pharmaceutical preparations by using HPLC with diode array (DAD) and UV detections [9,10].

Nowadays, the availability of high performance analytical procedure for sample treatment in clinical analytical chemistry, allowing the sensitive and selective quantification of the target active principle (for TDM purposes) is still a critical point and the limiting step in terms of time and solvent consuption. In this scenario, the new sample treatment procedures are required for biological matrices in order to reduce these drawbacks.

Fabric pahse sorptive extraction (FPSE), an innovative sample preparation technique as a part of sorbent-based extraction and microextraction techniques [11], provides these advantages for several matrices, from biological samples [12-18] to food/food supplements [19, 20], and also for cosmetic and environmental one [21, 22].

Recently, this technology was also succesfully applied for *in vivo non invasive* exhaled breath aereosol (EBA) sampling to monitor the human exposure to xenobiotics [16] or to simplify the whole procedure as valid and superior alternative to the Dried Blood Spot (DBS) [23].

The herein proposed and validated procedure shows a simple analytical method using FPSE-based sample preparation coupled with a rugged and well-known instrument configuration based on HPLC with UV detection, for the quantification of FVP in human plasma and breast milk.

2. Materials and Methods

2.1 Chemicals and reagents

The sol-gel polycaprolactone-polydimethylsiloxane-polycaprolactone (sol-gel PCAP-PDMS-PCAP) sorbent coated FPSE membrane was obtained by the Department of Chemistry and Biochemistry of Florida International University (Miami, Florida, USA). Fabric substrate unbleached Muslin, 100% cellulose cotton, was purchased from JoAnn Fabrics (Miami, Florida, USA), while triblock co-polymer poly(caprolactone)-block-poly(dimethylsiloxane)-block-poly(caprolactone) (PCAP-PDMS-PCAP) and sol-gel precursor methyl trimethoxysilane (MTMS) were purchased from Gelest Inc. (Morrisville, PA, USA). Sodium hydroxide and hydrochloric acid were purchased from Thermofisher Scientific (Milwaukee, WI, USA). Trifluoroacetic acid, acetone, methanol, methylene chloride, and ammonium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The chemical standard of FVP was purchased from Shanghai Yingxuan Pharmaceutical Science & Technology (China). Acetonitrile, methanol and orthophosphoric acid (HPLC grade) were purchased from Merck (Darmstadt, Germany), and the water was purified using a Human Ultra Water Purification System (Japan).

2.2 Preparation of standard solutions

A stock solution of FVP (0.1 mg/mL) was prepared and diluted with methanol to give working standard solutions from 0.2 to 50 µg/mL. The stock and working solutions were stored at +4°C and proved to be stable for the entire study.

2.3 Preparation of sol-gel PCAP-PDMS-PCAP coated fabric phase sorptive extraction membrane

Preparation of sol-gel PCAP-PDMS-PCAP coated FPSE membrane involves four distinct and sequential steps: (a) selection and surface preparation of the fabric substrate to maximize the sol-gel sorbent loading; (b) preparation of the sol solution to prepare the sol-gel coating on the surface treated fabric substrate; (c) sol-gel sorbent coating on the substrate for a predetermined time; and (d) onditioning, aging, and cleaning of the sol-gel sorbent coated FPSE membranes [24]. Considering the high polarity of FVP, hydrophilic 100% cellulose cotton fabric was selected as the best FPSE substrate candidate. Sol solution was prepared by sequential addition and subsequent thorough mixing of 5.0 g of polycaprolactone-block-polydimethylsiloxane-block-polycaprolactone polymer, 5 mL methyl trimethoxysilane (MTMS), 10 mL acetone:methylene chloride (50:50, *v:v*), and 2.5 mL trifluoroacetic acid (5% water) in a 50-mL centrifuge tube. The sol solution was vortexed for 3 min, and finally the sol solution was centrifuged at 14000 rpm for 3 min. The supernatant was transferred into an amber glass reaction vessel, and a 20 cm x 10 cm treated cellulose fabric was immersed into the sol solution. The sol-gel sorbent coating process continued under dip coating technique for 4 h at ambient temperature and pressure. Subsequently, the sol-gel sorbent–coated membrane was withdrawn from the reaction vessel, and dried in a desiccator for 1 h. The sol-gel sorbent coated FPSE membrane was then conditioned and aged for 24 h at 50°C in a homemade conditioning device for 24 h at 50°C under continuous nitrogen gas flow. After conditioning, the FPSE membrane was washed with methanol:methylene chloride (50:50, *v:v*) mixed solvent system for 30 min under sonication, and after drying in air for 15 min, the membrane was further processed for 12 h in the conditioning device at 50°C under continuous nitrogen gas flow. The sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane was then cut into 1.0 cm x 1.0 cm and stored in an air-tight container to keep it clean until its application in bioanalysis.

2.4 Instrumentation

The stock standard solutions and working standard solutions were prepared using a Fisher Scientific digital vortex mixer (Fisher Scientific, USA) and an Eppendorf Centrifuge Model 5415 R (Eppendorf North America Inc. USA). In order to obtain solutions without air bubbles, a BRANSON 2510 ultrasound (Branson Inc., USA) was used, while for the ultrapure deionized water used in the sol-gel synthesis phase, the Barnstead NANOPure Diamond deionized water (Model D11911, Dubuque, IA) system was used.

A Perkin Elmer Spectrum 100 FT-IR spectrometer with universal ATR (Santa Clara, CA) was used for the FT-IR characterization of the fabric substrates and coated FPSE

membranes, while an electron microscope Philips XL scan 30 equipped with an EDAX detector was used for the Scanning Electron Microscopy (SEM) analysis.

The HPLC analyses were performed using a Shimadzu (Japan) LC 20 liquid chromatograph system (LC-20AT pump, SIL-20A HT autosampler, RF-20A UV detector). During the method development, different chromatographic parameters and mobile phase compositions were evaluated. For the separation different columns like Spherisorb ODS1 C18 column (Waters Spa, Milford, MA, USA; 4.6 mm × 150 mm, 5 µm), Shim-Pack C18 (ODS) column (Shimadzu Corporation-Japan; 150 mm x 4.6 mm x 5 µm), and Luna C18 (ODS) column (Phenomenex, Torrance, CA, USA; 4.6 mm × 150 mm, 5 µm), different mobile phase compositions, different temperatures (25°C, 35°C, 40°C, and 45°C) and different flow rates (from 0.5 to 1.5 mL/min.) were evaluated. The chromatographic column was thermostated using a CTO-10AS VP oven, while the quantitative analyses were performed at 315 nm, corresponding to the FVP maximum wavelenght.

The better performances in terms of sensitivity (signal to noise ratio, S/N), overall runtime and absence of interferences, were obtained using the C18 (ODS) Shim-Pack column (150 mm x 4.6 mm x 5 µm) thermostated at 30ºC (±1°C) and using a mobile phase composed by acetonitrile-10 mM orthophosphoric acid (25:75, *v:v*) under isocratic conditions at 0.8 mL/min. The wavelength was set at 315 nm. The chromatograms related to blank matrices and FVP spiked samples are showed in **Figure 1**.

Figure 1

2.5 Optimization of FPSE procedure

Venous blood samples were collected from peripheral veins of a volunteer (informed consent form was obtained according to Ethical Commitee approval, E.21700) into tubes containing disodium EDTA and centrifuged at 4500 × *g* for 10 min. The resultant plasma samples were stored at −20°C until analysis. Breast milk samples were collected from a 35 years old volunteer mum (informed consent form was obtained according to Ethical Commitee approval, E.21700) into polyethylene storage packs. The breast milk samples were stored at −20°C. To extract the drug from both matrices, FPSE technique was used through the following steps: *i)* the membranes were cut into squares of 1 cm^2 of surface; *ii)* the FPSE membrane was cleaned and activated by methanol: water mixture (60:40, *v:v*); *iii)* the FPSE membranes were rinsed in deionized water; *iv)* the FPSE membranes were immersed into 500 µL of plasma and breast milk spiked with working solutions of FVP; *v)* the extraction was

carried out under magnetic stirring at 200 rpm at room temperature; *vi)* the back-extraction was carried out using methanol as back extraction solvent; *vii)* 20 µL of the extract were directly injected into the chromatographic system.

2.6 FPSE-HPLC-UV method validation

The procedure herein reported was validated according to the International Conference on Harmonization (ICH) Guidelines [25] for analytical figure of merits such as: selectivity, trueness, precision, linearity, LOD, LOQ, ruggedness, and stability.

3. Results and Discussion

3.1 Selection of the FPSE sorbent chemistry

Favipiravir is a very polar analyte with log Kow value ~-0.6. As a result, it is very affinitive towards water, highlighting the challenges related to the isolation and preconcentration of polar analytes from aqueous samples. Unlike classical sample preparation techniques, fabric phase sorptive extraction (FPSE) utilizes its three building blocks, a fabric substrate (hydrophilic/hydrophobic), a chemical linker that combines the sol-gel polymer network to the fabric substrate, and an organic polymer/organic-inorganic copolymer that provides different intermolecular interactions via its functional groups to the target analytes.

All these blocks allow an highly affinitive extraction membrane. Other great advantage is related to the planar geometry that expands and enhance the surface area for the intermolecular interactions with the target analytes and consequently allow fast extraction kinetics. Due to the built-in porosity of the substrate, FPSE combines the extraction principles of solid phase microextraction (SPME) and solid phase extraction (SPE) by design. As a result, FPSE offer exhaustive or near exhaustive extraction of the target analytes even if the extraction is carried out under direct immersion extraction mode (similar to a SPME fiber). The polymer used in the current study was poly(caprolactone)-block-poly(dimethylsiloxane)-block-poly(caprolactone) and is a medium polar polymer made up of polar poly(caprolactone) segments and nonpolar poly(dimethyl siloxane). Methyl trimethoxysilane (MTMS) was used as a linker in order to use additional London dispersion type weak intermolecular interaction to the target analyte via its methyl pendant group. The presence of abundant surface hydroxyl group in cellulose makes it an ideal substrate to binds the sol-gel sorbent during the sol-gel coating process, while the residual hydroxyl groups on cellulose substrate after the sol-gel sorbent coating may allow the interaction with the target analyte also via hydrogen bonding.

3.2 Characterization of FPSE membrane

The sol-gel PCAP-PDMS-PCAP coated FPSE membranes as well as the substrate, 100% cellulose cotton were characterized using Scanning Electron Microscopy (SEM) and Fourier Transform-Infrared Spectroscopy (FT-IR).

3.2.1 Scanning Electron Spectroscopy

Figure 2 reports the SEM images of (a) uncoated 100% cellulose cotton fabric and (b) sol-gel PCAP-PDMS-PCAP coated FPSE membrane, both at 1,000x magnifications. The SEM image of uncoated fabric substrate demonstrates the individual microfibril of cellulose fabric with clear spacing among them. The sol-gel PCAP-PDMS-PCAP sorbent coating presented in **Figure 2(b)** appears to be uniformly distributed on the microfibrils. The through pores of the fabric substrate becomes narrower after the sol-gel sorbent coating, however, the sol-gel PCAP-PDMS-PCAP coated FPSE membranes are still permeable without requiring any external pressure.

Figure 2

3.2.2 Fourier Transform-Infrared Spectroscopy (FT-IR)

FT-IR spectra are presented in **Figure 3(a)**. **Figure 3(b)** represents the FT-IR spectra sol-gel PCAP-PDMS-PCAP coated FPSE membrane. The FT-IR spectra of other two building blocks are presented in the Supplementary document. Major characrertisic bands in the FT-IR spectra of PCAP-PDMS-PCAP includes 1082 cm^{-1} which may be assigned to the stretching vibration of Si-O bonds of the triblock copolymer. The stretching vibration of carbonyl group is seen at 1723 cm^{-1}, which corresponds to the ester structural unit in polycaprolactone subchain [26]. Bands at 2946 cm^{-1} and 2864 cm^{-1} represent asymmetric and symmetric CH_2 stretching, respectively [27].

The noteworthy bands of the FT-IR spectra of uncoated cellulose fabric include CH_2 asymmetric and symmetric streching at 2913 cm^{-1} and 2848 cm^{-1}, respectively. The band at 1431 cm^{-1} represents CH_2 sissoring, 1314 cm^{-1} represents CH_2 rocking, 1243 cm^{-1} may correspond to C=O stretching, 1155 cm^{-1} may be assigned to anti-symmetrical bridge C-O-C stretching and 1063 cm^{-1} represents C-O stretching [28,29].

The characteritic bands of the FT-IR spectra of MTMS include 1264 cm^{-1} and 789 cm^{-1} that represent Si-CH_3 group. The band at 1138 cm^{-1} represents the –CH_3 rocking modes of the methoxy group [30,31].

Figure 3

3.3 Optimized FPSE procedure

To extract the drug from both matrices efficiently, FPSE technique was used through the following steps: *i)* the membranes were cut into squares of 1 cm^2 of surface; *ii)* the FPSE membrane was cleaned and activated by methanol: water (60:40, *v:v*) for 5 min; *iii)* the FPSE membranes were rinsed in deionized water; *iv)* the FPSE membranes were immersed into dilution of 350 µL of plasma and breast milk spiked with working solutions of FVP, with 200 µL serum physiologic (0.9% NaCI); *v)* the extraction was carried out under magnetic stirring at 200 rpm at room temperature for 30 min; *vi)* the back-extraction (500 µL) was carried out by using methanol for 30 min; *vii)* 20 µL of the extract were injected into the chromatographic system.

In order to achieve the maximum analyte recovery, the FPSE procedure optimization and related results were reported in ***Supplementary materials, section S.1***.

3.4 Chromatographic process

Various mobile phases, column types, and column sizes combinations were tested with different flow rates and column temperatures. RP-HPLC was preffered and C18 (ODS) column (4.6 mm I.D, 150 mm length and 5 µm particle size) was used with mobile phase composed of acetonitrile-10 mM orthophosphoric acid (25:75, *v:v*) under isocratic conditions with flow rate of 0.8 mL/min at 30ºC (±1°C) to achieve the highest resolution and reproducibility analyses. The retention times are 3.9±0.1 min. and 4.1±0.1 min. for plasma and breast milk, respectively (for the HPLC performances see **Table 1**).

In addition, one of the major advantages of the present method lies in the fact that the chromatographic run takes place under isocratic elution conditions. In this way the method is immediately transferable from one instrument to another as the main chromatographic parameters (as indicated in **Table 1**) are maintained. This aspect is of great importance especially in application fields where routine analyses are performed and operators are often not experts in the field of analytical chemistry and the transferability of analysis methods.

3.5 Method Validation

3.5.1 Linearity

Calibration curves (n=6), obtained using linear least-squares regression analysis by plotting of peak areas *versus* the corresponding FVP concentrations, show correlation

coefficient of 0.9985 and 0.9981 for plasma and breast milk, respectively. For plasma assays the method is linear over the range 0.2-50 µg/mL, while for breast milk assays the linearity is observed and validated in the range 0.5-25 µg/mL. The figure of merits for the analytical performance of the validated method are summarized in **Table 1**.

Table 1

3.5.2 Sensitivity

The limit of detection (LOD) and limit of quantitation (LOQ) were determined as reported in [25], and the results were reported in **Table 1**.

3.5.3 Precision and trueness

Precision and trueness were determined by the analysis of QC samples at three concentration levels of 1, 10, 50 µg/mL for plasma, and 1, 10, 20 µg/mL for breast milk (low, medium and high QC levels). The trueness, expressed by recovery (as allowed by the International Guidelines) was determined with standard addition method by spiking QC samples of standard drug solutions to plasma and breast milk including 10 µg/mL of FVP. The precision (intraday and interday) was reported in terms of RSD% values. Recovery values were found higher than 86.0% in both matrices, as reported in **Table 2**.

Table 2

To evaluate the precision of the method, intraday and interday repeatability were investigated by calculating the RSD% of five replicates at each concentration on the same day (intraday precision) and on three different days (interday precision). The intraday and interday RSD% values are less than 4.2% for both matrices, as reported in **Table 3**.

Table 3

3.5.4 Ruggedness

Ruggedness was evaluated by making small but deliberated changes on the optimized conditions on the flow-rate, column oven temperature and mobile phase composition. The mobile phase proportions were changed from (25:75, *v:v*) (acetonitrile-acidic solution) to 30:70 and 20:80; column temperature was changed from 30ºC to 25ºC and 35ºC; and the flow

rate which is 0.8 ml/min originally was changed from 0.6 to 1.0 mL/min. These changes had no significant effect on peak areas, and on chromatographic parameters, as reported in **Table 4**.

Table 4

3.5.5 Stability

The effects of freeze and thawn steps on FVP were studied in both matrices using spiked QC samples, and evaluated also at 24 h and 8 h at room temperature, and at –20°C for 4 weeks. No changes in the chromatographic elution profiles and in terms of FVP concentration was observed.

3.6 Real sample analysis

The herein validated method was applied to a real sample of 32-year-old lactating mother with COVID-19 and took Favimol® 200 mg tablets (Neutec Pharmaceutical Company) after a single oral dose and following the prescribed protocol. 5 mL of venous blood samples and 5 mL breast milk were collected after drug administration. The blood and breast milk samples were then processed according to the herein described and validated method. The obtained results, reported in **Table 5**, highlight the applicability of the procedure for TDM purposes, while in **Figure 4** were reported the chromatograms related to real sample analysis.

Table 5

Figure 4

4. Conclusion

FVP is an antiviral drug used to treat cases of influenza that are resistant to conventional treatments, and after COVID-19 pandemic, it is allowed to use at high doses up to 1600 mg/day to treat the life threatening infection. However, none of the professional societes and organizational guidelines (IDSA guidelines, World Health Organization guidelines, National Institutes of Health guidelines) recommend the use of FVP in the management of COVID-19, due to the varying results of existing clinical trials data [32]. Due to the fact that the data containing at least one FVP-related outcome or side effects were

considered sufficient, and the lack of data on the effects in infants, the quantitation of FVP in plasma and breast milk is nowadays important. In the proposed method a simple analytical procedure (FPSE combined to HPLC), has been developed and validated for FVP quantification in human plasma and breast milk. Sample preparation starting from biological matrices (e.g. blood, urine, breast milk, etc.) is still challenging, time consuming, multi-step and often incurs high percentage of analyte loss. As herein highlighted FPSE has addressed most of the shortcomings of classical sample preparation techniques and established itself as a total sample preparation solution for bioanalytical chemistry, confirmed in the present work by the applicability of the combined FPSE-HPLC-UV/Vis method to therapeutic drug monitoring. Furthermore, one of the advantages of the present method lies in the fact that the chromatographic run takes place under isocratic elution conditions making easy to transfer the procedure from one instrument to another.

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.

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Figure and Table Captions

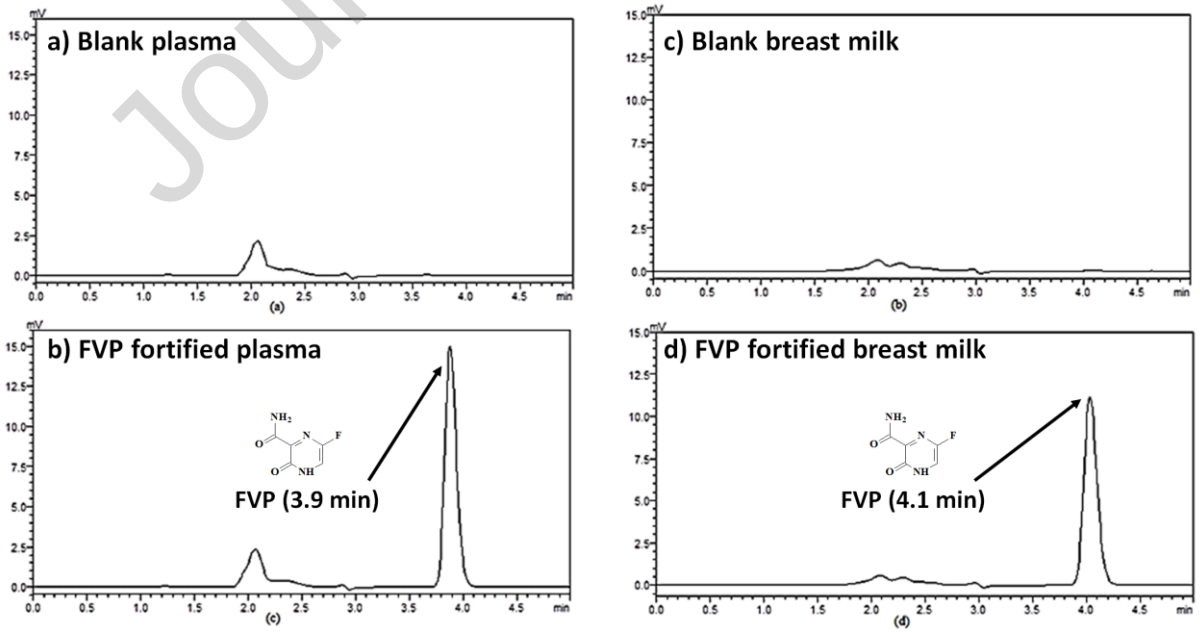

Figure 1. The chromatograms of (a) blank plasma, (b) blank breast milk, (c) 50 µg/mL FVP spiked plasma, and (d) 25 µg/mL FVP spiked breast milk.

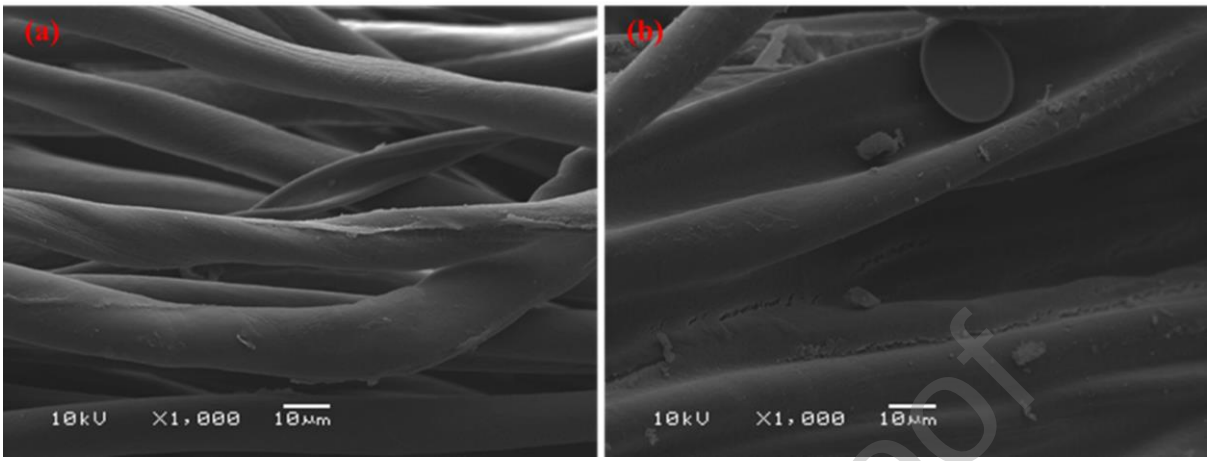

Figure 2. SEM images of (a) uncoated cellulose fabric at 1,000x magnifications, and (b) sol-gel PCAP-PDMS-PCAP coated FPSE membrane at 1,000x magnifications.

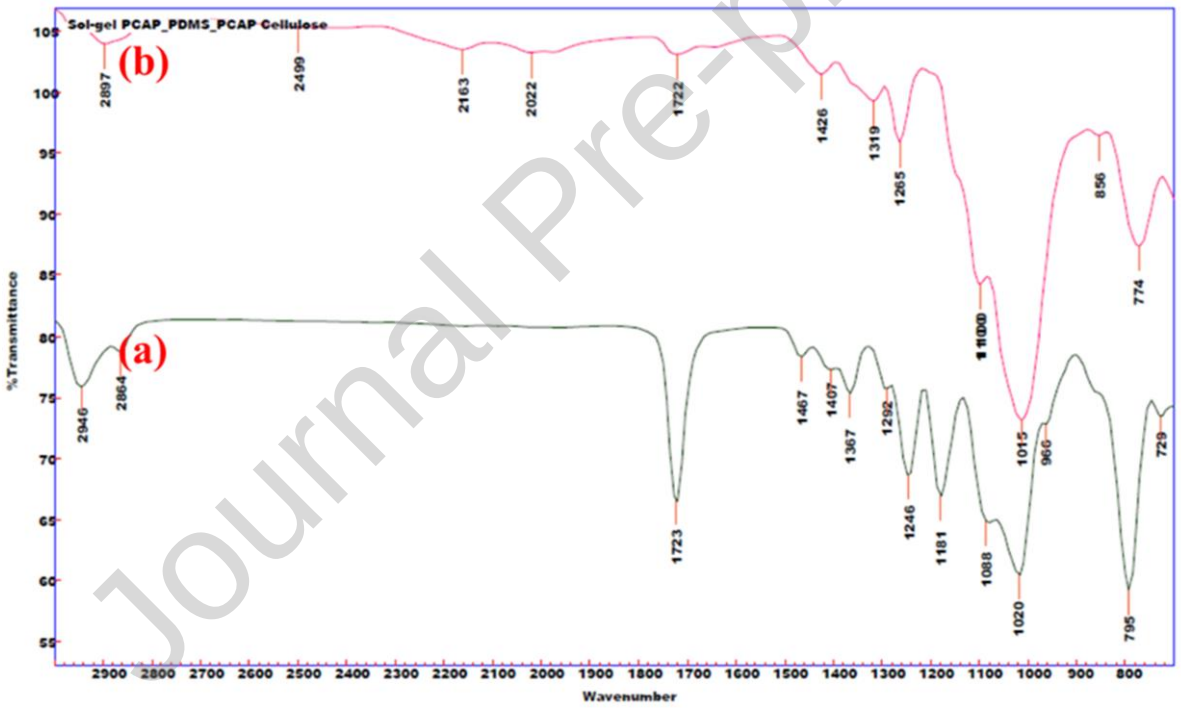

Figure 3. FT-IR spectra of (a) pristine poly(caprolactone)-b-poly(dimethylsiloxane)-b-poly(caprolactone) polymer; (b) sol-gel PCAPPDMS- PCAP sorbent coated FPSE membrane.

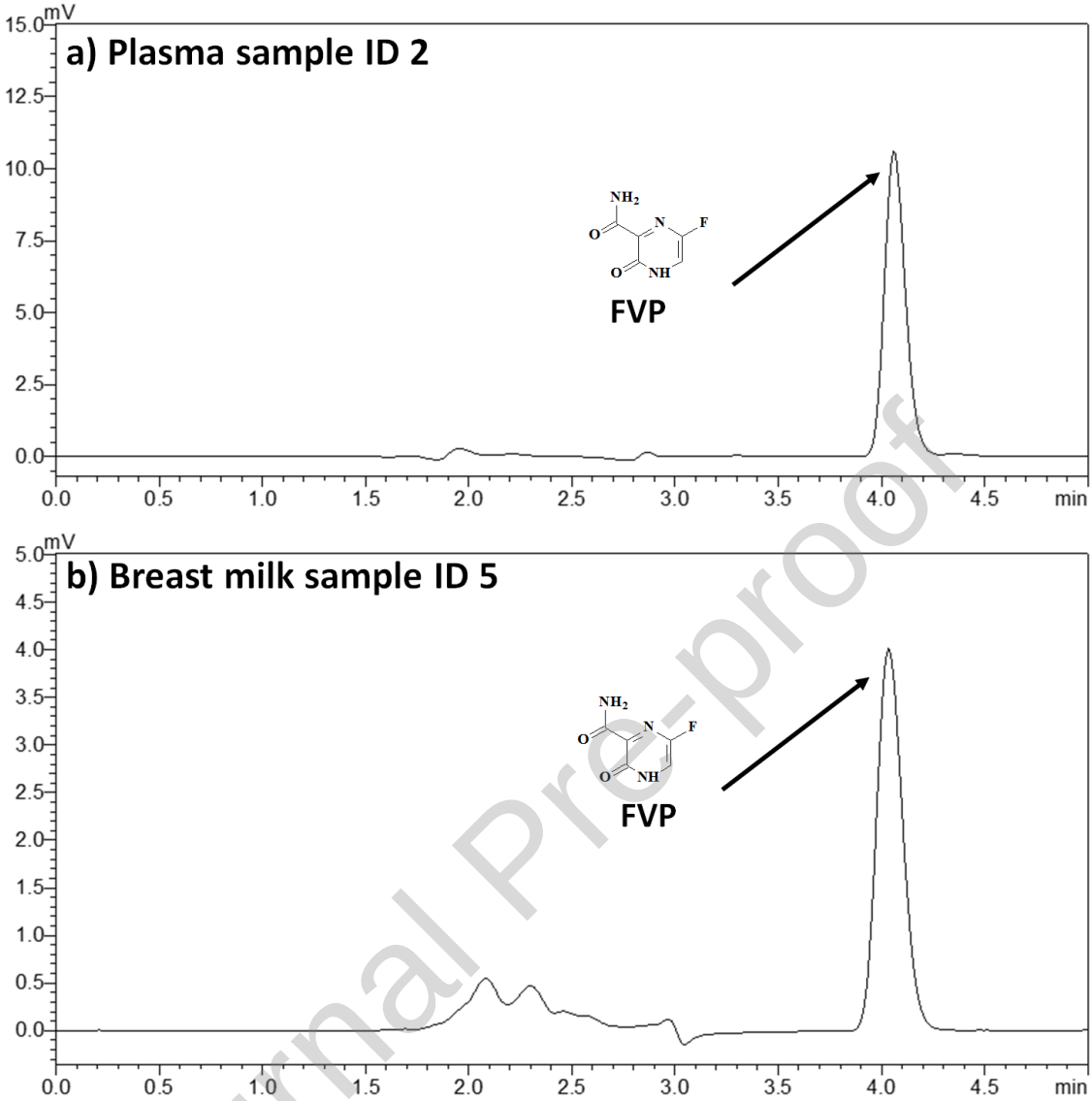

Figure 4. Chromatograms of the real sample analyses. Related to a) plasma sample (ID 2, Table 5); b) breast milk sample (ID 5, Table 5)

Table 1 Chromatographic system suitability parameters and results of analytical parameters of the proposed method.
Table 2 Results of Recovery Studies by Standard Addition Method.
Table 3 Precision of the method.
Table 4 Ruggedness of the method.
Table 5 Real sample analysis to monitor FVP in plasma and breast milk.

Table 1 Chromatographic system suitability parameters and results of analytical parameters of the proposed method

Parameters	**Plasma**	**Breast Milk**
Resolution	8.7	6.9
HETP	0.05	0.08

N*	5600±90	4450±70
Tailing factor*	1.3±0.1	1.2±0.1
Asymmetry Factor*	1.2±0.1	0.9±0.1
Retention time (min)	3.9±0.1	4.1±0.1
Concentration range[a] (µg/mL)	0.2-50	0.5-25
Regression equation[b]	y=20441x+670	y=25498x+551
Intercept±SD	670±16	551±25
Slope±SD	20441±96	25498±101
Correlation coefficient (r^2)	0.9985	0.9981
LOD (µg/mL)	0.06	0.15
LOQ (µg/mL)	0.2	0.5

*mean values of the parameters are mentioned; [a] n=6; [b] y = xC + b where C is the concentration in µg mL^{-1} and y is the peak area

Table 2 Results of Recovery Studies by Standard Addition Method

	Amount added (µg/mL)	**Total amount found (µg/mL)**	**Recovery (%)**	**RSD (%)**
plasma	11	10.3	93.6	2.9
	20	17.2	86.0	3.7
	60	56.5	94.2	2.4
breast milk	11	9.9	90.0	3.5
	20	17.3	86.5	3.7
	30	28.4	94.7	4.2

n=5

Table 3 Precision of the method

	Studied concentration (µg/mL)	RSD (%) Intraday variation	RSD (%) Interday variation
plasma	1	1.0	3.2
	10	1.0	2.9
	50	2.1	2.5
breast milk	1	0.9	1.2
	10	1.2	2.2
	20	1.5	3.6

n=5

Table 4 Ruggedness of the method

Condition	Value	Recovery (%)		RSD (%)	
		plasma	breast milk	plasma	breast milk
flow rate mL/min	0.8	96.7	86.5	0.2	0.2
	0.6	91.2	105.2	1.2	2.5
	1.0	89.7	102.3	1.3	2.2
mobile phase ratio	25:75	96.7	86.3	0.2	0.2
	20:80	89.7	100.7	3.9	2.7
	30:70	91.2	102.3	4.1	3.8
column temperature	30	96.7	86.3	0.2	0.2
	25	93.1	101.4	3.5	3.1
	35	94.3	102.3	2.9	2.91

The gray boxes represent the optimized and validated conditions. n=3

Table 5 Real sample analysis to monitor FVP in plasma and breast milk

ID	Matrix	Age	Treatment (mg/day)	Time (h) after last drug assumption	FVP concentration (µg/mL)
1	plasma	32	200	0.5	6.4
2		32	200	2	2.9
3		32	200	4	1.3
4	Breast milk	32	200	0.5	0.3
5		32	200	2	5.5
6		32	200	4	2.7

CRediT authorship contribution statement

Gizem Tiris: Investigation, Writing – review & editing. **Isil Gazioglu**: Conceptualization, Validation, Supervision, Methodology, Writing – review & editing. **Kenneth G. Furton**: Investigation, Writing – review & editing. **Abuzar Kabir**: Investigation, Methodology, Writing – review & editing. **Marcello Locatelli:** Methodology, Writing – original draft, Writing – review & editing.

Declaration of interests

☐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.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Highlights

- Favipiravir is an antiviral drug used to treat cases of influenza that are resistant to conventional treatments.
- After COVID-19 pandemic, according to the clinical trials, it became a promising antiviral agent for corona virus.
- The proposed and validated procedure shows a simple analytical method utilizing FPSE-based sample preparation.
- Well-known instrument configuration based on HPLC with UV detection, for the quantification of FVP in human plasma and breast milk.