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Simultaneous determination of febuxostat and montelukast in human plasma using fabric phase sorptive extraction and high performance liquid chromatography-fluorometric detection

--Manuscript Draft--

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Abstract:	In the present work, a new sensitive and selective high-performance liquid chromatography-fluorimetric detection (HPLC-FLD) method was developed and validated to quantify febuxostat (FBX) and montelukast (MON) in human plasma. The developed procedure was successfully applied to a study aimed at evaluating the pharmacokinetic profiles of febuxostat and montelukast in human plasma. A sol-gel poly (caprolactone)- block -poly(dimethylsiloxane)- block -poly(caprolactone) (sol-gel PCAP-PDMS-PCAP) extraction sorbent coated fabric phase sorptive extraction membrane was used in the extraction process. The entire chromatographic analysis was performed with isocratic elution of the composition of the mobile phase (acetonitrile:water, 60:40, v:v , 0.032% glacial acetic acid) on the C18 column. The flow rate is varied during the analysis, particularly from 0.5 mL min -1 at the start and linearly increased to 1.5 mL min -1 in 7 min. The detection and quantification of the analytes was carried out by means of a fluorimetric detector at 320 nm and 350 nm as absorption wavelengths and at 380 and 400 nm as emission wavelengths for FBX and MON, respectively.The calibration curves demonstrated linearity in the range 0.3-10 ng mL -1 and 5-100 ng mL -1 for FBX and MON, respectively, while the LOD and LOQ values were 0.1 and 0.3 ng mL -1 for FBX and 1.5 and 5 ng mL -1 for MON. Intraday and interday RSD% values were found lower than 5.79%. As reported, the method was applied to real plasma samples obtained from a volunteer who was co-administered both the drugs. Pharmacokinetic data reveal that the concentration of both the drugs reaches the plateau approximately at the same time, but exhibits an elimination phase at different rates. This study demonstrated the usefulness of the new method and its applicability in therapeutic drug monitoring (TDM).
Suggested Reviewers:	Imran Ali Taibah University drimran.chiral@gmail.com expertise in analytical approaches for quantitative analyses
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	Victoria Samanidou Aristotle University of Thessaloniki: Aristoteleio Panepistemio Thessalonikes samanidu@chem.auth.gr expertise in extraction and quantification of xenobiotics and pharmacokinetic

	determination

Dear Editor,

Enclosed please find the manuscript “Simultaneous determination of febuxostat and montelukast in human plasma using fabric phase sorptive extraction and high performance liquid chromatography-fluorometric detection” submitted to **Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences**, as full lenght article.

In this work, we report a novel and sensitive high performance liquid chromatography-fluorimetric detection (HPLC-FLD) method for the determination of febuxostat (FBX) and montelukast (MON) in human plasma and subsequently applied to a pharmacokinetic study
The analytes were extracted from plasma using sol-gel poly(caprolactone)-block-poly (dimethylsiloxane)-block-poly(caprolactone)(sol-gel PCAP-PDMS-PCAP) coated fabric phase sorptive extraction membrane.

The whole procedure has been validated agreeing to the ICH,

This new approach, included the use of sol-gel PCAP-PDMS-PCAP sorbent coating on 100% cellulose cotton fabric prepared by sequential mixing and subsequent vigorous mixing of poly(caprolactone)-b-poly(dimethylsiloxane)-b-poly(caprolactone) polymer as FPSE membrane and C18 as the stationary phase used in HPLC, exhibits high potential for immediate adaptation as a rapid, robust and environmental friendly tool for diagnostic and analytical, clinical purposes.

The developed and validated method applied to a real sample from a volunteer who was administered both drugs at the same time. The pharmakokinetic data reveal that the concentration of both the drugs reach the plateu in blood at about the sametime but drop down at different rates. This prototype study proved the utility of the new method.

The reported manuscript fit with journal aims because it included sampling (extraction optimization), separations, and instrumentation measurements (method development). The purposed method offers an original application of FPSE.

We hope, the manuscript is prepared according to the journal rules and will be considered for evaluation for the publication possibility in the “Journal of Chromatography B”.

Sincerely,
Isil Gazioglu
Bezmialem Vakif University,
Faculty of Pharmacy, Department of Analytical Chemistry,
Fatih 34093 Istanbul, Turkey.

Dear Editor

Enclosed please find the revised manuscript "**Simultaneous determination of febuxostat and montelukast in human plasma using fabric phase sorptive extraction and high performance liquid chromatography-fluorimetric detection**", submitted to **Journal of Chromatography B**.

We thank the Editor and the Reviewers for their very positive evaluations and for the suggestions that were all accepted and reported in the revised version. All changes were highlighted in the "track changes" files, as required, and were reported the point-by-point responses.

The reported manuscript fit with journal aims because it included sampling, HPLC separations, and innovative multi-analytes procedure in biological matrix.

The manuscript has been read and approved by all authors. The authors declare that does not exist any economic interest or any conflict of interest.

This research has not been disclosed or published and is not under consideration for publication elsewhere.

Sincerely,

Isil Gazioglu

Editor

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following major revision. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Jan 10, 2022.

The Authors thank the Editor for the evaluation and final decision to give us the opportunity to improve the quality of the work based on the recommendations of the Reviewers.

All suggestions have been accepted and reported in the revised version. Specifically, the changes made are indicated in the "track changes" files.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

All suggestions have been accepted and reported in the revised version. Specifically, the changes made are indicated in the "track changes" files.

Reviewer 1

The manuscript is on an interesting topic and deserves publication after some...changes as shown below:

The Authors thank the Reviewer for the evaluation, for indicating that it reports interesting topic and deserves publication, and for the excellent suggestions. All recommendations have been accepted and reported in the revised version. Specifically, the changes made are indicated in the "track changes" files.

ABSTRACT "The whole chromatographic analysis, carried out on a C18 stationary phase using acetonitrile: water (60:40, v:v, 0.032% glacial acetic acid) as the mobile phase in an isocratic elution with a variable flow rate from 0.5 mL min-1 at the beginning and increased up to at 1.5 mL min-1 in 7 min. The detection and quantification was performed at absorption at 320 and 350 nm FBX and MON, with emission wavelengths at 380 and 400 nm for FBX and MON, respectively". This sentence is not really clear, please rephrase.

Accordingly to the Reviewer comment, the sentences were revised in "The entire chromatographic analysis was performed with isocratic elution of the composition of the mobile phase (acetonitrile:water, 60:40, v:v, 0.032% glacial acetic acid) on the C18 column. The flow rate is varied during the analysis, particularly from 0.5 mL min^{-1} at the start and linearly increased to 1.5 mL min^{-1} in 7 min. The detection and quantification of the analytes was carried out by means of fluorimetric detectors at 320 nm and 350 nm as absorption wavelengths and at 380 and 400 nm as emission wavelengths for FBX and MON, respectively", in order to better clarify the procedure.

Page 7 Line 7: "the extraction was carried out under magnetic stirring at 200 rpm at room temperature; vii) the retro-extraction of the analytes from the membranes was carried out by means of a small volume of organic solvent". Important details were not reported regarding the extraction process. The extraction times? The retro-extraction solvent? And time? Please, specify. Please, describe better the extraction process optimization.

Accordingly to the Reviewer comment, these information were added.

Page 11 Line 54 (section 3.4): In the section "Chromatographic process" more details regarding the optimization of chromatographic separation should be reported.

Accordingly to the Reviewer comment, these information were added. Section 3.4 was renamed as "Optimization of the chromatographic separation".

Page 12 Line 19 (section 3.4.1): The section regarding the validation should be revisited: the section "Preparing calibration curve and calculation of method sensitivity" should be named differently. Data regarding linearity of the method were included in this Section (not sensitivity). Data regarding the Ruggedness of the method should be also report (for example Retention factor and selectivity α for optimized parameters and for changed parameters).

Accordingly to the Reviewer comment, the section was revised. As also suggested by the Reviewer 2, paragraphs 3.4.1, 3.4.2, 3.4.3, and 3.5 were coupled in a single section named "3.5 Analytical figure of merits" in order to highlight all the validation parameters in a single section. Regarding the ruggedness, Table 3 was added with the requested information on retention factor and selectivity.

Reviewer 2

The manuscript describes an analytical method to evaluate the concentration of febuxostat and montelukast in human plasma. The rationale was provided and the experimental sections are well-organized. I suggest to address the following criticisms in order to improve the quality of the paper:
The authors thank the Reviewer for the evaluation, for indicating that rationale was provided and the experimental sections are well-organized, and for the excellent suggestions. All recommendations have been accepted and reported in the revised version. Specifically, the changes made are indicated in the "track changes" files.

Paragraphs 3.4.1, 3.4.2, 3.4.3, and 3.5 should be coupled in a single section;
As suggested, the four paragraphs were coupled in a single section named "3.5 Analytical figure of merits" in order to highlight all the validation parameters in a single section.

Figures 3 and 4 should be coupled in a single figure with the following caption: "SEM images and FT-IR spectra of the FPSE membranes used in the present validated method". Consequently also the text need to be revised;
Accordingly to the right suggestion, the figures 3 and 4 were coupled in a single figure (number 3 in the revised version). Consequently the old figure 5 was renumbered as figure 4. Also the caption was revised accordingly in: "Figure 3. SEM images and FT-IR spectra of the FPSE membranes used in the present validated method: (a) SEM of uncoated cellulose fabric at 100x magnifications; (b) SEM of sol-gel PCAP-PDMS-PCAP coated FPSE membrane at 100x magnifications; (c) SEM of sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane at 5,000x magnifications; (d) FT-IR spectra of Methyltrimethoxysilane; (e) FT-IR spectra of pristine poly(caprolactone)-b-poly(dimethylsiloxane)-b-poly(caprolactone) polymer; (f) FT-IR spectra of sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane".

Accordingly to the previous comment, I suggest to couple the paragraphs 3.2, 3.2.1, and 3.2.2 in a single section named "characterization of FPSE membrane";
As suggested, the three paragraphs were coupled in a single section named "3.2 Characterization of FPSE membrane"

Check references list accordingly to Authors guidelines (e.g. the pages of ref 33 were not reported);
As suggested, the references list was revised accordingly to Authors guidelines.

Check the caption of figure 4: "beta" should be used instead of "b".
The caption was not revised because the letter "b" is right, as also reported in https://pubs.acs.org/doi/abs/10.1021/bm0100054. In this case the letter "b" is referred to the term "block". As requested by Reviewer, figures 3 and 4 were coupled in a single figure and this revision was also reported.

Highlights

- A novel and sensitive FPSE-HPLC-FLD method for the determination of febuxostat and montelukast in human plasma.
- Direct analysis of human plasma sample.
- Synergistic effect on sensitivity and selectivity from FPSE-HPLC-FLD configuration.
- First FPSE application for the detection of febuxostat and montelukast.

ABSTRACT

In the present work, a new sensitive and selective high-performance liquid chromatography - fluorometric detection (HPLC-FLD) method was validated to quantify the febuxostat (FBX) and montelukast (MON) in human plasma. The developed procedure was then successfully applied to a study aimed at evaluating the pharmacokinetic profiles of febuxostat and montelukast in human plasma. A sol-gel poly (caprolactone)-block-poly(dimethylsiloxane)-block-poly(caprolactone) (sol-gel PCAP-PDMS-PCAP) extraction sorbent coated fabric phase membrane was used in the plasma extraction process. The whole chromatographic analysis, carried out on a C18 stationary phase using acetonitrile: water (60:40, *v:v*, 0.032% glacial acetic acid) as the mobile phase in an isocratic elution with a variable flow rate from 0.5 mL min^{-1} at the beginning and increased up to at 1.5 mL min^{-1} in 7 min.The detection and quantification was performed at absorption at 320 and 350 nm FBX and MON, with emission wavelengths at 380 and 400 nm for FBX and MON, respectively.

The calibration curves demonstrated linearity in the range 0.3-10 ng mL^{-1} and 5-100 ng mL^{-1} for FBX and MON, respectively, while the LOD and LOQ values were 0.1 and 0.3 ng mL^{-1} for FBX and 1.5 and 5 ng mL^{-1} for MON. During the validation process, intraday and interday RSD% values were found lower than 5.79%. As reported, the method was then applied to a real plasma sample obtained from a volunteer who was administered both the drugs at the same time. Pharmacokinetic data reveal that the concentration of both the drugs reaches the plateau approximately at the same time, but exhibits an elimination phase at different rates. This study demonstrated the usefulness of the new method and its applicability in therapeutic drug monitoring (TDM).

ABSTRACT

In the present work, a new sensitive and selective high-performance liquid chromatography-fluorimetric detection (HPLC-FLD) method was developed and validated to quantify febuxostat (FBX) and montelukast (MON) in human plasma. The developed procedure was successfully applied to a study aimed at evaluating the pharmacokinetic profiles of febuxostat and montelukast in human plasma. A sol-gel poly (caprolactone)-*block*-poly(dimethylsiloxane)-*block*-poly(caprolactone) (sol-gel PCAP-PDMS-PCAP) extraction sorbent coated fabric phase sorptive extraction membrane was used in the extraction process. The entire chromatographic analysis was performed with isocratic elution of the composition of the mobile phase (acetonitrile:water, 60:40, *v:v*, 0.032% glacial acetic acid) on the C18 column. The flow rate is varied during the analysis, particularly from 0.5 mL min^{-1} at the start and linearly increased to 1.5 mL min^{-1} in 7 min. The detection and quantification of the analytes was carried out by means of a fluorimetric detector at 320 nm and 350 nm as absorption wavelengths and at 380 and 400 nm as emission wavelengths for FBX and MON, respectively.The calibration curves demonstrated linearity in the range 0.3-10 ng mL^{-1} and 5-100 ng mL^{-1} for FBX and MON, respectively, while the LOD and LOQ values were 0.1 and 0.3 ng mL^{-1} for FBX and 1.5 and 5 ng mL^{-1} for MON. Intraday and interday RSD% values were found lower than 5.79%. As reported, the method was applied to real plasma samples obtained from a volunteer who was co-administered both the drugs. Pharmacokinetic data reveal that the concentration of both the drugs reaches the plateau approximately at the same time, but exhibits an elimination phase at different rates. This study demonstrated the usefulness of the new method and its applicability in therapeutic drug monitoring (TDM).

Simultaneous determination of febuxostat and montelukast in human plasma using fabric phase sorptive extraction and high performance liquid chromatography-fluorometric detection

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ABSTRACT

In the present work, a new sensitive and selective high-performance liquid chromatography - fluorometric detection (HPLC-FLD) method was validated to quantify the febuxostat (FBX) and montelukast (MON) in human plasma. The developed procedure was then successfully applied to a study aimed at evaluating the pharmacokinetic profiles of febuxostat and montelukast in human plasma. A sol-gel poly (caprolactone)-block-poly(dimethylsiloxane)-block-poly(caprolactone) (sol-gel PCAP-PDMS-PCAP) extraction sorbent coated fabric phase membrane was used in the plasma extraction process. The whole chromatographic analysis, carried out on a C18 stationary phase using acetonitrile: water (60:40, *v:v*, 0.032% glacial acetic acid) as the mobile phase in an isocratic elution with a variable flow rate from 0.5 mL min^{-1} at the beginning and increased up to at 1.5 mL min^{-1} in 7 min.The detection and quantification was performed at absorption at 320 and 350 nm FBX and MON, with emission wavelengths at 380 and 400 nm for FBX and MON, respectively.

The calibration curves demonstrated linearity in the range 0.3-10 ng mL^{-1} and 5-100 ng mL^{-1} for FBX and MON, respectively, while the LOD and LOQ values were 0.1 and 0.3 ng mL^{-1} for FBX and 1.5 and 5 ng mL^{-1} for MON. During the validation process, intraday and interday RSD% values were found lower than 5.79%. As reported, the method was then applied to a real plasma sample obtained from a volunteer who was administered both the drugs at the same time. Pharmacokinetic data reveal that the concentration of both the drugs reaches the plateau approximately at the same time, but exhibits an elimination phase at different rates. This study demonstrated the usefulness of the new method and its applicability in therapeutic drug monitoring (TDM).

Keywords: Febuxostat, Montelukast, Fabric Phase Sorptive Extraction, HPLC, Plasma Analysis

1. Introduction

Febuxostat (FBX), commercial name of 2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid, is a drug used nowadays in the treatment of gout that plays its function by decreasing the amount of uric acid in the blood. It represents the treatment of choice, especially if allopurinol is not adequate (or not usable) for this treatment [1]. The safety profile of this drug in terms of side effects are extensively studied and reported in the literature [2-6]. Montelukast (MON), commercial name of [R-(E)]-1-[[[[1-[3-[2-(7-chloro-2-quinolinyl) ethenyl]phenyl]-3-[2-(7-chlor-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl]propyl]thio]methyl cyclopropane acetic acid sodium, is used as a modifier of leukotrienes widely used in case of bronchoconstriction and asthma [7]. This drug is often found in combination with others and for this reason its safety profile and its effectiveness can vary, making therapeutic monitoring essential in order to maintain its therapeutic effect. The chemical structures of FBX and MON are shown in **Figure 1**.

Following the increasingly frequent clinical practice of administering drug combinations [8], monitoring their presence in biological matrix has become increasingly important in recent years and at the same time nessesiates the development of new validated methods to meet the increasing demand of clinical practice.

To date, there are only two HPLC-based pharmacokinetic (PK) studies for FBX in rat plasma samples in the literature [9,10], and only a few methods for analysis in human plasma with LC-MS / MS [11-14]. Various HPLC methods for the determination of MON in plasma with UV detection or tandem mass spectrometry are reported in the literature [7,15-18]. Only one work reports the simultaneous determination of such drugs in human plasma [19] and is based on high performance thin layer chromatography (HPTLC) combined with fluorescence detection. Furthermore, in the reported study, no real sample was analyzed, but only samples of an "*ad-hoc*" spiked biological matrix to verify the applicability of the procedure.

It should also be noted that most of the reported methods consider the precipitation of proteins prior to the liquid-liquid extraction targetted to extract the analytes from the biological matrix.

Continuing our research on the characterization and application of fabric phase sorptive extraction (FPSE) on complex matrices [20-27], the objective of the current bioanalytical methodology is to synergistically exploit the high selectivity of the sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane in efficiently isolating FBX and MON from human plasma with detection and quantification in high performance liquid chromatography hyphenated with a highly sensitive and selective fluorimetric detector (HPLC-FLD).

The method was then applied to a real plasma sample obtained from a volunteer who was administered both the drugs at the same time. The entire process was approved by the Ethical Committee and Pharmacokinetic data reveal that the concentration of both drugs reaches the plateau approximately at the same time, but exhibits an elimination phase at different rates. This study demonstrated the usefulness of the new method and its applicability in therapeutic drug monitoring (TDM).

2. Experimental

2.1. Chemicals and Reagents

The sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane was synthesized at the Department of Chemistry and Biochemistry of Florida International University (Miami, Florida, USA). In the FPSE membrane synthesis process, methyl trimethoxysilane (MTMS), acetone, trifluoroacetic acid (TFA) and dichloromethane were purchased from Sigma-Aldrich (St. Louis, MO, USA), while PCAP-PDMS-PCAP was purchased from Gelest Inc. (Morrisville, PA, USA). Sodium hydroxide, and hydrochloric acid was supplied by Thermo Fisher Scientific

(Milwaukee, WI, USA). Unbleached muslin (100% cotton) membrane substrates were purchased from Jo-Ann Fabric (Miami, FL, USA).

The chemical standards of FBX and MON (analytical grade, purity ≥99%) were purchased from Shanghai Yingxuan Pharmaceutical Science & Technology (China), while the drugs for human use Adenuric® (80 mg of FBX) and Singulair® (5 mg MON), were bought in a local pharmacy (Istanbul, Turkey). Acetonitrile, methanol, and glacial acetic acid (HPLC grade, Merck, Darmstadt, Germany) were used without further purification steps, and the water was purified using a Human Ultra Water Purification System (Japan).

2.2. Preparation of standard solutions

The stock solutions of the two analytes FBX and MON at a concentration of 0.1 mg mL^{-1} were obtained by solubilizing the chemical standards in methanol and subsequently diluted to obtain working solutions from 0.1 to 10 ng mL^{-1} (FBX) and 5 to 100 ng mL^{-1} (MON). The stock and working solutions were stored at 4 °C and proved stable for the duration of the study.

2.3. 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, a system of Barnstead NANOPure Diamond deionized water (Model D11911, Dubuque, IA). 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 was used for the SEM analysis. Philips XL scan 30 equipped with an EDAX detector.

The HPLC analyzes were performed using a Shimadzu (Japan) LC 20 liquid chromatograph equipped with LC-20AT pump, SIL-20A HT autosampler, RF-20A fluorimetric detector (set to wavelengths of 320 and 350 nm for excitation, and 380 and 400 nm for the emission of FBX and MON, respectively). The chromatographic column was thermostated at room temperature using a CTO-10AS VP oven and the resolution of the two analytes was obtained using a Phenomenex Luna C18 (ODS) column (4.6 mm x 125 mm, 5 µm).

The entire chromatographic analysis, carried out using acetonitrile: water (60:40, *v:v*, 0.032% of glacial acetic acid) as the mobile phase in an isocratic elution (in terms of percentage composition of the phase) with a varying flow rate from 0.5 mL min^{-1} in the beginning and increased to 1.5 mL min^{-1} in 7 min. Under these conditions, the entire chromatographic run took 13 min., allowing the resolution of FBX and MON without any matrix interference. A representative chromatogram obtained from plasma spiked with 50 ng/mL of the target analytes is shown in **Figure 2**.

2.4. Sample preparation and extraction

The plasma samples obtained from blood draws from the peripheral veins of the volunteers (5 mL) used in the present study, provided by the blood bank of the Bezmialem Vakif University Hospital of the Istanbul Faculty of Medicine (Turkey, ethics committee approval, No. 15/10) were stored in test tubes containing disodium EDTA and centrifuged at 4500 × *g* for 10 min. and kept at -20°C until analysis.

For the extraction process of FBX and MON the 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: acetonitrile (40:60, *v:v*) for 5 minutes; *iii)* the FPSE membranes were rinsed in deionized water; *iv)* 20 µL of plasma were diluted with 280 µL of isotonic solution (0.9% sodium chloride); *v)* 100 µL portions of standard solutions

at different concentrations (for the calibration curve and the QC quality control samples) were added for each drug; *vi)* the extraction was carried out under magnetic stirring at 200 rpm at room temperature; *vii)* the retro-extraction of the analytes from the membranes was carried out by means of a small volume of organic solvent; *viii)* finally 10 µL of the extract were injected into the chromatographic system.

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

In the preparation of the sol solution for the creation of the PCAP-PDMS-PCAP sol-gel coating on 100% cotton fabric, poly(caprolactone)-block-poly(dimethylsiloxane)-block-poly(caprolactone) polymer was mixed under vortex for 3 min., methyl trimethoxysilane, methylene chloride, trifluoroacetic acid and deionized water in a 50 mL reaction vessel with a molar ratio of 0.07:1:94:2.3:0.75:3, respectively, to ensure a homogeneous mixture. In the solution thus obtained, sonicated for 10 min., a cellulose fabric (15 cm x 45 cm) is immersed to thicken the sol-gel coating through a process carried out at room temperature for 8 h. At the end, the membrane was removed, dried in air for 1 h, and subjected to heat treatment at 50°C for 24 h under a continuous flow of nitrogen gas. The obtained FPSE membrane was then washed with a 50:50 (*v:v*) mixture of methylene chloride and methanol under sonication for 1 h and treated again at 50°C for 12 h. The FPSE membrane coated with sol-gel PCAP-PDMS-PCAP sorbent was then stored in an airtight container until its use for characterization and application in plasma sample preparation.

2.6. Validation of the method

The method reported here has been validated according to the International Conference on Harmonization (ICH) and CDER Guidelines [28,29] for analytical parameters such as: linearity, precision and trueness (both intraday and interday), LOD, LOQ, robustness and stability.

3. Results and discussion

3.1. Selection of the FPSE sorbent chemistry

Compared to the conventional (micro) extraction procedures based on sorbents, in the case of FPSE, a functionalized three-dimensional network is created and suitable for maximizing the retention of the analytes on *(a)* a fabric substrate that not only hosts the network of sol- gel sorbent, but also contributes to the overall selectivity of the FPSE membrane via its intrinsic hydrophilic/hydrophobic attribute; *(b)* a sol-gel precursor which acts as a bridge between the fabric substrate and the sol-gel sorbent network; *(c)* an organic/inorganic polymer that provides the primary interaction between the analyte(s) and the membrane via its diversified functional groups.

The major advantage of this technique lies in the fact that it allows to reconcile the principles of exhaustive extraction (typical of solid phase extraction, SPE) with the principles of equilibrium extraction (typical of solid phase microextraction, SPME).

In the present work, given that the two analytes considered show a large difference in polarity (polar mean FBX, and non-polar MON), the greatest analytical challenge was the creation and selection of a phase capable of extracting these compounds with maximum efficiency.

In the present work the objective was achieved through the use of an FPSE membrane containing sol-gel poly (caprolactone)-block-poly(dimethylsiloxane)-block-poly(caprolactone). In fact, in this phase, poly(caprolactone) is a polar, biocompatible and biodegradable polymer. Poly(dimethylsiloxane) is a non-polar and uniquely biocompatible

polymer. The simultaneous presence of both functionalities made the polymer an ideal extraction sorbent for the current analytical challenge.

In the polymer synthesis phase, methyl trimethoxysilane was selected as a sol-gel precursor for its terminal methyl group which can exert an additional interaction of the London dispersion type towards the analytes during the FPSE process, but also because in the sol-gel synthesis it represents one of the best available linkers.

The selected substrate (cotton muslin, 100% cellulose) derives from the fact that it shows an intrinsic hydrophilic character, essential for obtaining a transfer of the aqueous sample matrix in the immediate vicinity of the sol-gel sorbent such as to maximize the interaction of the analytes with the sol-gel PCAP-PDMS-PCAP through different intermolecular interactions.

3.2. *Characterization of FPSE membrane*

The FPSE membranes were characterized by Fourier transform infrared spectroscopy analysis techniques and by scanning electron microscopy techniques, based on what reported below.

3.2.1. Scanning Electron Microscopy

Scanning electron microscopy (SEM) allows elucidating the surface morphology of the sol-gel sorbent coating by revealing information about the uniformity of the coating. Given the principles on which the technique is based, the possibility of having a substrate available with distinct pores even after the sol-gel coating process is of fundamental importance.

Figure 3 shows the SEM images of *(a)* substrate of uncoated cellulose fabric at 100x magnification respectively; *(b)* FPSE membrane coated with PCAP-PDMS-PCAP sol-gel sorbent at 100x magnification; and *(c)* PCAP-PDMS-PCAP sol-gel coated FPSE membrane at 5000 magnification.

Following this analysis, it is observed that the FPSE membrane reveals the presence of unique pores of the cellulose fabric substrate which are preserved even after coating. At the same time, it is observed that the sol-gel sorbent coating is uniformly distributed on the substrate and that any differences between the uncoated and coated fabric are difficult to recognize. A further magnification (**Figure 3c**) allows to reveal how the absorbent is distributed uniformly around the single microfibril of cellulose with consequent presence of a rough surface of the sol-gel coating.

3.2.2. Fourier Transform Infrared Spectroscopy

Based on the FT-IR analysis reported in **Figure 4 (ac)** and referring to the main constituent elements of the FPSE membrane, methyltrimeoxysilane, poly(caprolactone)-block--poly (dimethylsiloxane)-block-poly (caprolactone) and the coated FPSE membranes coated with sol-gel PCAP-PDMS-PCAP sorbent, it is possible to observe in **(a)** the characteristic bands at 1265 cm^{-1} and 788 cm^{-1} relating to the vibration of CH_3 group of the precursor [30], while the signals at 1188 cm^{-1} and 1076 cm^{-1} are attributable to the CO stretching of Si-O-CH_3. The signals at 2840 cm^{-1} and 1465 cm^{-1} can be assigned to the C-H stretching and bending vibration of Si-O-CH3, respectively [31]. Noteworthy bands obtained from the polymer poly(caprolactone)-block-poly(dimethylsiloxane)-block-poly (caprolactone) include the stretching of the carbonyl at 1722 cm^{-1}, the asymmetric stretching of CH_2 at 2949 cm^{-1}, the symmetrical stretch of CH_2 at 2865 cm^{-1}, CO stretching at 1292 cm^{-1}. There is also a strong signal at 795 cm^{-1} which could be attributed to CH_3 rocking originating from the PDMS block of the polymer, while that at 1246 cm^{-1} to the symmetrical folding of Si(CH_3), a characteristic feature of the PDMS block [32]. Many bands appeared simultaneously in both MTMS and poly(caprolactone)-block-poly (dimethylsiloxane)-block-poly (caprolactone) and the sol-gel PCAP-PDMS-PCAP sorbent

coated FPSE membrane strongly suggests successful integration of both the components in the FPSE membrane.

3.3. Optimization of FPSE procedure

To obtain the best performance from the FPSE extraction procedure, it was necessary to optimize various conditions, such as: *i)* extraction times*; ii)* back-extraction time; *iii)* type of back-extraction/elution solvent; *iv)* back-extraction solvent volume. The various conditions tested are: from 5 to 60 min. for extraction and back-extraction; acetonitrile, acetonitrile: methanol (40:60, *v:v*), acetonitrile: water (60:40, *v:v*), methanol, methanol: water (60:40, *v:v*) as back-extraction solvents with different volumes.

Furthermore, as reported in the literature [33,34], in order to reduce the process times, the extraction by vortexing at 200 rpm, room temperature for 30 min 20 µL of plasma diluted with 280 µL of physiological (0.9% NaCI) solution was also tested. Following the various conditions tested, the optimum procedure was found to be: *i)* the membranes were cut into squares of 1 cm2 of surface; *ii)* the FPSE membrane was cleaned and activated by methanol: acetonitrile (40:60, *v:v*) for 5 minutes; *iii)* the FPSE membranes were rinsed in deionized water*; iv)* 20 µL of plasma were diluted with 280 µL of isotonic solution (0.9% sodium chloride); *v)* 100 µL portions of standard solutions at different concentrations (for the calibration curve and the QC quality control samples) were added for each drug; *vi)* the extraction was carried out under magnetic stirring at 200 rpm at room temperature; *vii)* the retro-extraction of the analytes from the membranes was carried out by means of a small volume of organic solvent; *viii)* finally 10 µL of the extract were injected into the chromatographic system.

3.4. Chromatographic process

The chromatographic analysis with the optimized conditions previously reported allowed the complete resolution of the analytes in 13 min. reaching a high selectivity as the presence of matrix interferers is not observed.

Figure 5 (ac) shows respectively the chromatographic profile of the blank matrix, and the chromatograms obtained from the analysis of blank plasma added with 5 ng mL^{-1} for FBX and 50 ng mL^{-1} for MON, and the chromatograms obtained from the healthy volunteer after 3 hours of drug administration.

3.4.1. Preparing calibration curve and calculation of method sensitivity

The validated calibration curves of the method herein reported were obtained by least squares linear regression analysis by plotting the peak areas of the analytes *vs.* concentration levels. The mean equation of the calibration curve (n = 6) obtained on seven concentration levels were: y = 120867x- 54315 (correlation coefficient = 0.9947) for FBX, and y = 49420x-335712 (correlation coefficient = 0.9959) for MON. All the parameters related to the analytical method performance are shown in **Table 1**.

3.4.2 Trueness, precision and recovery

Both intraday and interday precision and trueness were evaluated by analyzing samples for quality control (QC) at three different concentrations (0.3, 5, 10 ng mL^{-1} for FBX and 5, 50 and 100 ng mL^{-1} for MON) and classified as low, medium and high concentration. The analyses were carried out in sixfold (n = 6) in the blank matrix and in the aqueous sample. Final recoveries of FBX and MON from the biological matrix were evaluated by extraction of spiked plasma samples and compared with the peak areas obtained from the same quantities of unextracted aqueous solutions of the drugs. The mean absolute recovery observed stood at

values of 89.2 and 92.3%, while the relative recoveries were 95.9 and 95.5% for FBX and MON, respectively.

Each test was performed both on the same day for intraday variability assessment and on six different days for interday precision (intermediate precision) and trueness. The RSD% values of the intraday and interday analyzes were all less than 5.79%. All these results have been summarized in **Table 2**.

3.4.3. Ruggedness

The robustness of the method, understood as the reproducibility of the method following small but deliberate variations in method parameters, was evaluated at different flow values, temperatures of the column furnace and different ratios of the mobile phase content. In these tests, the percentage composition of the mobile phase was varied from 60:40 to 50:50 and 25:75. The temperature of the column was changed from 40ºC to 35ºC and 45ºC, while the flow from 0.5 to 1.5 in 7 min was ranged from 0.8 to 1.8 and from 0.3 to 1.2 mL min^{-1} over the same time. The variations studied showed that the chromatographic conditions had no effect on the peak area and on the analytical performances of the procedure.

3.5. Stability study

During the validation process of the method, the stability of the analytes and any effects of the storage conditions on them were also evaluated. These studies were evaluated considering blank plasma samples added to the 3 QC levels and subjected to four freeze-thaw cycles before analysis, as well as monitoring stability at room temperature for 24 h, and at –20°C for 2 weeks. The stock solutions of FBX and MON were stable for at least 30 days when stored at -20ºC. After 30 days, no change in the chromatographic profiles of the analytes was observed. The

solutions were stable for 4 days if the samples were kept at 4°C using a sample cooler, 1 day at room temperature.

3.6. Application of the composite method to real plasma analysis

The validated method was then applied to the analysis of real samples to evaluate the pharmacokinetic profile (PK) on a healthy 24-year-old volunteer who was administered a single oral dose of 80 mg FBX and 15 mg MON. 5 mL of venous blood samples were collected prior to drug administration (baseline) and 1, 2, 3, 4, 6, 8, 10, and 12 h after the first day based on the pharmacokinetics of both drugs. The blood samples were then processed according to the method described above. Specifically, **Figure 2c** shows the chromatogram of the plasma sample obtained 3 h after administration. Since matrix stability was evaluated, the samples were stored at –20°C until analysis. The pharmacokinetic parameters were calculated using the analysis performed with this method to derive the area values under the plasma concentration-time curves ($AUC_{0\text{-}12}$, $AUC_{0\text{-}\infty}$) using the TOPFIT 2.0 pharmacodynamic and pharmacokinetic data analysis system. The plasma concentration-time curves of FBX and MON following oral administration of a single dose of 80 mg FBX and 15 mg MON are shown in **Figure 5**.

Conclusions

The method validated in the present work is able to simultaneously determine FBX and MON drugs widely used respectively for the treatment of gout and asthma, in plasma matrix. The method represents an excellent procedure for clinical applications aimed at therapeutic monitoring since, following the increase in clinical practice of using drug combinations, it is evident that obtaining the therapeutic effect, reducing/eliminating adverse effects, and above all the therapeutic window within well-defined concentration ranges, is of fundamental

importance especially when dealing with drugs that may have drug interactions among each other.

This method allows overall cost reduction, reduces analysis time, provides a simple analysis for simultaneous quantification in plasma samples with a superior selectivity and sensitivity to meet clinical needs. In the literature, there are some methods for the analysis of these substances separately, however there is only one method for the simultaneous dosing for both drugs in HPTLC. The advantages of this procedure mainly depend on the pretreatment method performed by the FPSE technique. This extraction approach is a relatively new and more efficient, environmentally friendly, and simpler technique than conventional extraction procedures such as liquid-liquid and solid-phase extraction techniques. After this simple pretreatment, reverse phase chromatography and fluorometric detection provided high sensitivity and selective analysis in the plasma samples. From this study it is clear that the presented method is a valid procedure for plasma analysis in patients undergoing such combination treatment.

Acknowlegments

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Declaration of Competing Interest

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

References

[1] H.R. Manasse, W.A. Zellmer, American Society of Health-System Pharmacists, Pharmacotherapy 21 (2001) 123-125.

[2] M.A. Becker, H.R. Schumacher, L.R. Espinoza, A.F. Wells, P. MacDonald, E. Lloyd, C. Lademacher, The urate-lowering efficacy and safety of febuxostat in the treatment of the hyperuricemia of gout: the CONFIRMS trial, Arthritis Res Ther 12 (2010).

[3] E. Roddy, M. Doherty, Epidemiology of gout, Arthritis Res Ther 12 (2010).

[4] I.S. Mackenzie, I. Ford, G. Nuki, J. Hallas, C.J. Hawkey, J. Webster, S.H. Ralston, M. Walters, M. Robertson, R. De Caterina, E. Findlay, F. Perez-Ruiz, J.J.V. McMurray, T.M. MacDonald, F.S. Grp, Long-term cardiovascular safety of febuxostat compared with allopurinol in patients with gout (FAST): a multicentre, prospective, randomised, open-label, non-inferiority trial, Lancet 396 (2020) 1745-1757.

[5] F.P. Ruiz, P. Richette, A.G. Stack, R.K. Gurunath, M.J.G. de Yebenes, L. Carmona, Failure to reach uric acid target of < 0.36 mmol/L in hyperuricaemia of gout is associated with elevated total and cardiovascular mortality, Rmd Open 5 (2019).

[6] W.B. White, K.G. Saag, M.A. Becker, J.S. Borer, P.B. Gorelick, A. Whelton, B. Hunt, M. Castillo, L. Gunawardhana, C. Investigators, Cardiovascular Safety of Febuxostat or Allopurinol in Patients with Gout, New Engl J Med 378 (2018) 1200-1210.

[7] S. Al-Rawithi, S. Al-Gazlan, W. Al-Ahmadi, I.A. Alshowaier, A. Yusuf, D.A. Raines, Expedient liquid chromatographic method with fluorescence detection for montelukast sodium in micro-samples of plasma, J Chromatogr B 754 (2001) 527-531.

[8] C. Celia, L. Di Marzio, M. Locatelli, P. Ramundo, F. D'Ambrosio, A. Tartaglia, Current trends in simultaneous determination of co-administered drugs, Separations 7(2) (2020), article 29, 1-18.

[9] B.B. Gabani, N.K. Saini, R.K. Jairam, P. Shrinivas, R.K. Trivedi, N.R. Srinivas, R. Mullangi, Simultaneous determination of colchicine and febuxostat in rat plasma: Application in a rat pharmacokinetic study, Biomed Chromatogr 34 (2020).

[10] D.G. Han, K.S. Kim, S.W. Seo, Y.M. Baek, Y. Jung, D.D. Kim, I.S. Yoon, A sensitive HPLC-FL method to simultaneously determine febuxostat and diclofenac in rat plasma: assessment of metabolic drug interactionsin vitroandin vivo, Anal Methods-Uk 12 (2020) 2166-2175.

[11] Y.C. Xu, J.L. Chen, D.D. Yang, Y. Hu, X.H. Hu, B. Jiang, Z.R. Ruan, H.G. Lou, Development of LC-MS/MS determination method and backpropagation artificial neural networks pharmacokinetic model of febuxostat in healthy subjects, J Clin Pharm Ther 46 (2021) 333-342.

[12] D. Tandel, P. Shah, K. Patel, V. Thakkar, K. Patel, T. Gandhi, Salting-Out Assisted Liquid-Liquid Extraction for Quantification of Febuxostat in Plasma Using RP-HPLC and Its Pharmacokinetic Application, J Chromatogr Sci 54 (2016) 1827-1833.

[13] B. Kamel, K.M. Williams, G.G. Graham, R.L.G. Norris, S.L. Stocker, J.E. Carland, K.D. Pile, R.O. Day, Determination of febuxostat in human plasma by high performance liquid chromatography (HPLC) with fluorescence-detection, Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 1126 (2019).

[14] F.A. El-Yazbi, O.A. Amin, E.I. El-Kimary, E.F. Khamis, S.E. Younis, M.A.W. Elkhatib, A.F. El-Yazbi, A novel HPLC-DAD method for simultaneous determination of febuxostat and diclofenac in biological samples: pharmacokinetic outcomes, Bioanalysis 11 (2019) 41-54.

[15] G.A. Smith, C.M. Rawls, R.L. Kunka, An automated method for the determination of montelukast in human plasma using dual-column HPLC analysis and peak height

summation of the parent compound and its photodegradation product, Pharm Res 21 (2004) 1539-1544.
[16] N.S. Rashed, Z.A. Nasr, Simultaneous Determination of Levocetirizine Dihydrochloride and Montelukast Sodium in Human Plasma by LC-MS/MS: Development, Validation, and Application to a Human Pharmacokinetic Study, Acta Chromatogr 31 (2019) 194-200.
[17] A.K. Shakya, T.A. Arafat, N.M. Hakooz, A.N. Abuawwad, H. Al-Hroub, M. Melhim, High-Performance Liquid Chromatographic Determination of Montelukast Sodium in Human Plasma: Application to Bioequivalence Study, Acta Chromatogr 26 (2014) 457-472.
[18] R. Muppavarapu, S. Guttikar, M. Rajappan, K. Kamarajan, R. Mullangi, Sensitive LC-MS/MS-ESI method for simultaneous determination of montelukast and fexofenadine in human plasma: application to a bioequivalence study, Biomed Chromatogr 28 (2014) 1048-1056.
[19] N.M. Hosny, N.N. Atia, S.M. El-Gizawy, D.M. Badary, M.S. Hareedy, Innovative HPTLC method with fluorescence detection for assessment of febuxostat-montelukast combination and study of their protective effects against gouty arthritis, Analyst 143 (2018) 4366-4378.
[20] A. Kabir, K.G. Furton, N. Tinari, L. Grossi, D. Innosa, D. Macerola, A. Tartaglia, V. Di Donato, C. D'Ovidio, M. Locatelli, Fabric Phase Sorptive Extraction-High Performance Liquid Chromatography-Photo Diode Array Detection Method for Simultaneous Monitoring of Three Inflammatory Bowel Disease Treatment Drugs in Whole Blood, Plasma and Urine, Journal Of Chromatography B 1084 (2018) 53-63.
[21] M. Locatelli, A. Tartaglia, F. D'Ambrosio, P. Ramundo, H.I. Ulusoy, K.G. Furton, A. Kabir, Biofluid sampler: A new gateway for mail-in-analysis of whole blood samples, Journal Of Chromatography B 1143 (2020) article 122055, 1-7.
[22] A. Tartaglia, A. Kabir, F. D'Ambrosio, P. Ramundo, S. Ulusoy, H.I. Ulusoy, G.M. Merone, F. Savini, C. D'Ovidio, U. De Grazia, K.G. Furton, M. Locatelli, Fast off-line FPSE-HPLC-PDA determination of six NSAIDs in saliva samples, Journal Of Chromatography B 1144 (2020) article 122082, 1-9.
[23] E. Agadellis, A. Tartaglia, M. Locatelli, A. Kabir, K.G. Furton, V. Samanidou, Mixed-mode fabric phase sorptive extraction of multiple tetracycline residues from milk samples prior to high performance liquid chromatography-ultraviolet analysis, Microchemical Journal, 159 (2020) article 105437, 1-10.
[24] M. Locatelli, A. Tartaglia, H.I. Ulusoy, S. Ulusoy, F. Savini, S. Rossi, F. Santavenere, G.M. Merone, E. Bassotti, C. D'Ovidio, E. Rosato, K. Furton, A. Kabir, Fabric phase sorptive membrane array as non-invasive in vivo sampling device for human exposure to different compounds, Analytical Chemistry 93(4) (2021) 1957-1961.
[25] M. Locatelli, N. Tinari, A. Grassadonia, A. Tartaglia, D. Macerola, S. Piccolantonio, E. Sperandio, C. D'Ovidio, S. Carradori, H.I. Ulusoy, K.G. Furton, A. Kabir, FPSE-HPLC-DAD method for the quantification of anticancer drugs in human whole blood, plasma and urine, Journal Of Chromatography B 1095 (2018) 204-213.
[26] I. Gazioglu, A. Kabir, O.S. Zengin, E.K. Tekkeli, K.G. Furton, A. Tartaglia, M. Locatelli, Development of sol-gel phenyl/methyl/poly (dimethylsiloxane) sorbent coating for fabric phase sorptive extraction and its application in monitoring human exposure to selected polycyclic aromatic hydrocarbons using high performance liquid chromatography-fluorescence detection, Journal Of Chromatography B 1163 (2021) article 112520, 1-8.
[27] I. Gazioglu, O. Sultan Zengin, A. Tartaglia, M. Locatelli, K.G. Furton, A. Kabir, Determination of polycyclic aromatic hydrocarbons (PAHs) in nutritional supplements

by fabric phase sorptive extraction (FPSE) with high-performance liquid chromatography (HPLC) with fluorescence detection, Analytical Letters 54(10) (2021) 1683-1696.
[28] CDER e CVM, Bioanalytical Method Validation-Guidance for Industry, Food and Drug Administration, May 2018.
[29] International Conference on Harmonization of Technical Requirements for registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline (2005) Validation of Analytical Procedures: Text and Methodology Q2(R1), Geneva, 2005.
[30] V. Samanidou, L.-D. Galanopoulos, A. Kabir, K.G. Furton, Fast extraction of amphenicols residues from raw milk using novel fabric phase sorptive extraction followed by high-performance liquid chromatography-diode array detection, Analytica Chimica Acta 855 (2015) 41-50. http://dx.doi.org/10.1016/j.aca.2014.11.036.
[31] B. Xu, J.J. Ge, Z.S. Cai, One-Step Processing to Fabricate Highly Transparent Superhydrophobic Surface via Granuliform Silica Aerogels, Advanced Materials Research 936 (2014) 1042-1046. 10.4028/www.scientific.net/AMR.936.1042.
[32] C. Gordin, M. Rusu, C. Delaite, S. Salhi, T. Elzein, M. Brogly, Crystalinity Behaviour in Poly(epsilon-caprolactone)-b-Poly(dimethylsiloxane) Diblock and Triblock Copolymers through FTIR and DSC, Mater. Plast. 46 (2009) 37-42.
[33] A. Kabir, M. Locatelli, H.I. Ulusoy, Recent Trends in Microextraction Techniques Employed in Analytical and Bioanalytical Sample Preparation, Separations 4 (2017).
[34] S. Gülle, H.I. Ulusoy, A. Kabir, A. Tartaglia, K.G. Furton, M. Locatelli, V.F. Samanidou, Application of fabric phase sorptive extraction-high performance liquid chromatography-photodiode array detection method for trace determination of Methylparaben, Propylparaben and Butylparaben in Cosmetic and Environmental Samples, Analytical Methods 11 (2019) 6136-6145.

Figure and Tables Captions

Figure 1: Chemical structures of FBX and MON

Figure 2: (a) Chromatogram of blank plasma, **(b)** The chromatograms obtained from plasma spiked with 5 ng mL^{-1} for FBX and 50 ng mL^{-1} for MON, (**c)** The chromatograms obtained from the healthy volunteer after 3 h of drugs administration

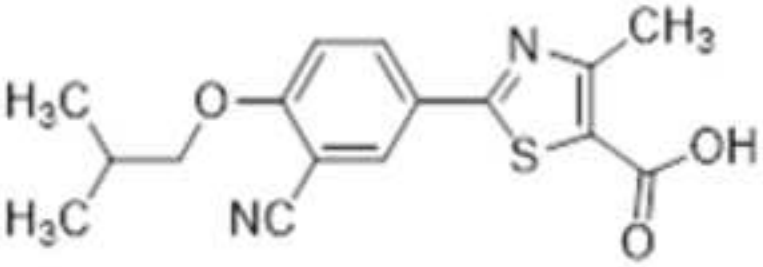
Figure 3. Scanning electron microscopy images of **(a)** uncoated cellulose fabric at 100x magnifications; **(b)** sol-gel PCAP-PDMS-PCAP coated FPSE membrane at 100x magnifications; **(c)** sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane at 5,000x magnifications.

Figure 4. FT-IR spectra of **(a)** Methyltrimethoxysilane; **(b)** pristine poly(caprolactone)-b-poly(dimethylsiloxane)-b-poly(caprolactone) polymer; **(c)** sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane.

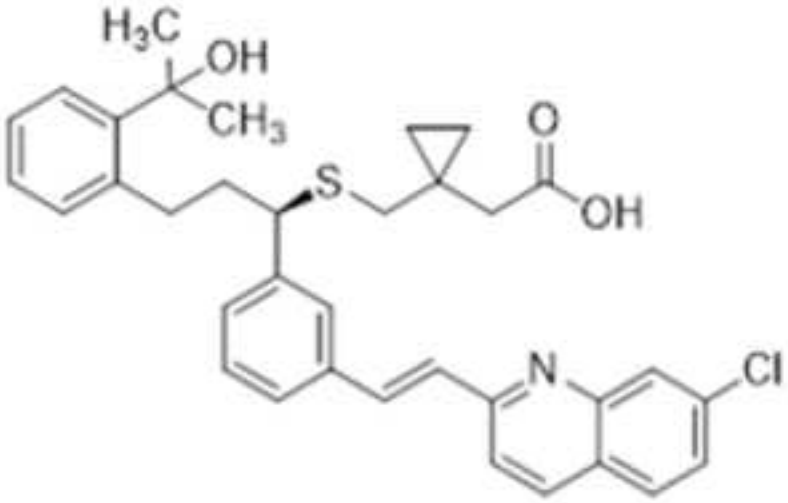
Figure 5. (**a)** Pharmacokinetic curve of FBX, (**b)** Pharmacokinetic curve of MON

Table 1. Analytical and chromatographic parameters of the method
Table 2. Precision and trueness of the method

Figure



Febuxostat



Montelukast

Figure

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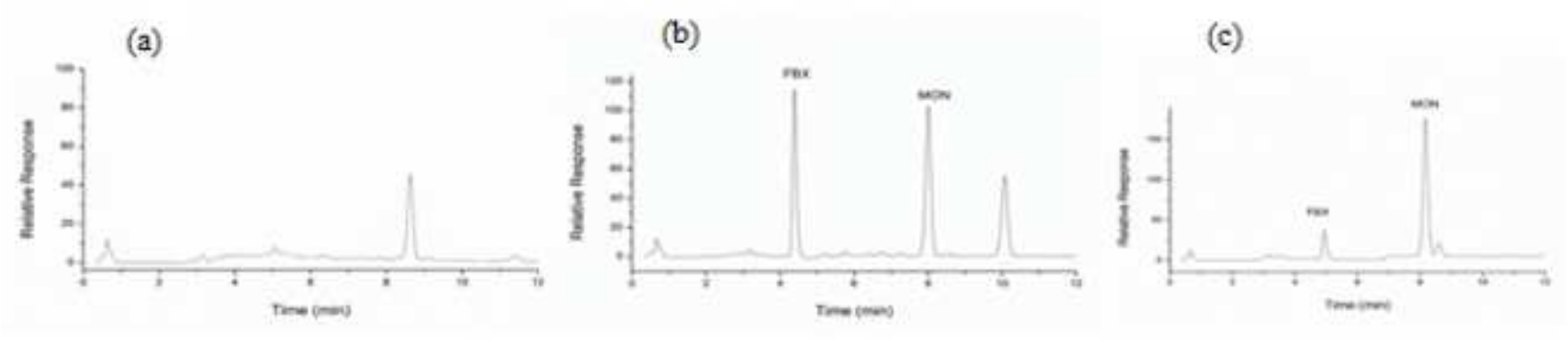

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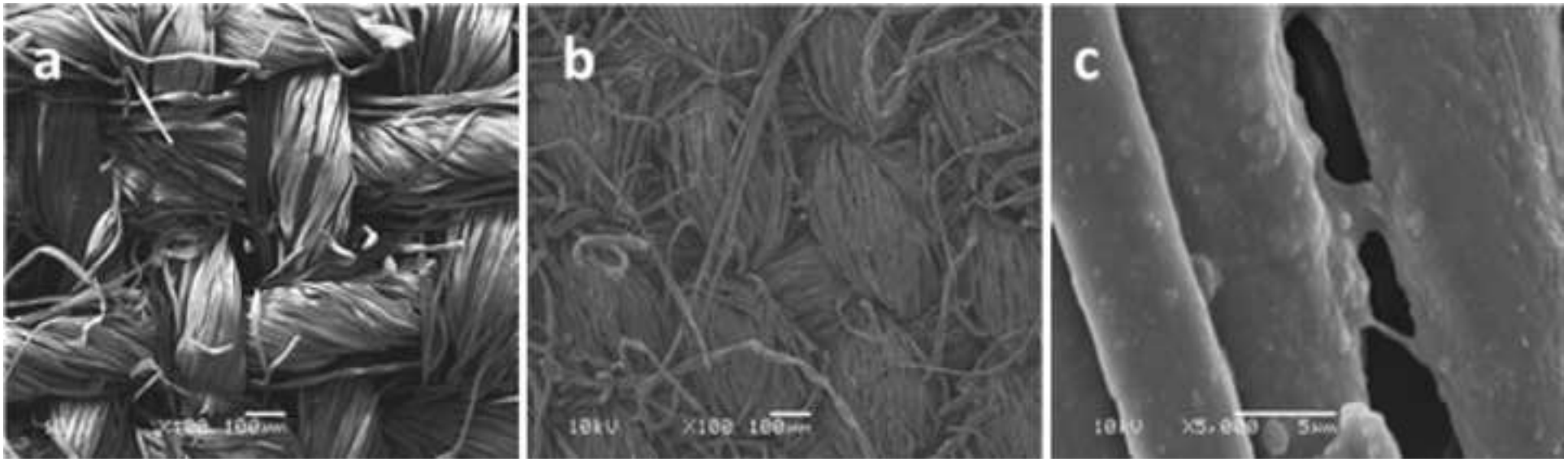

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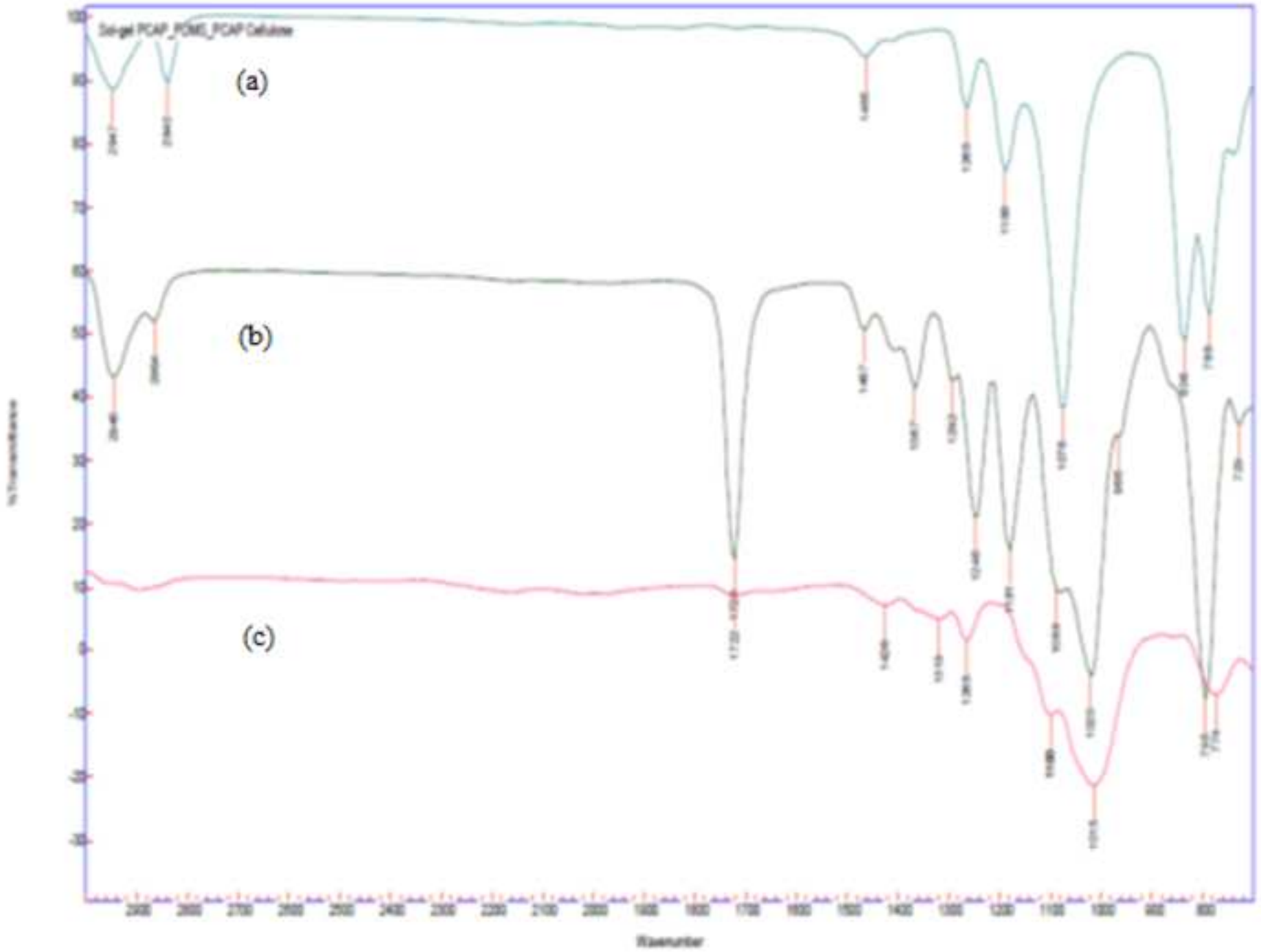

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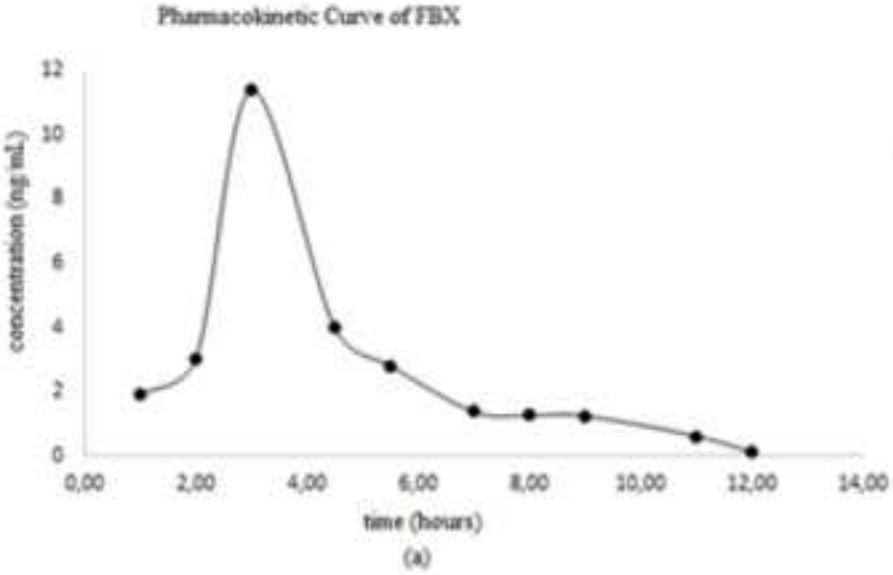
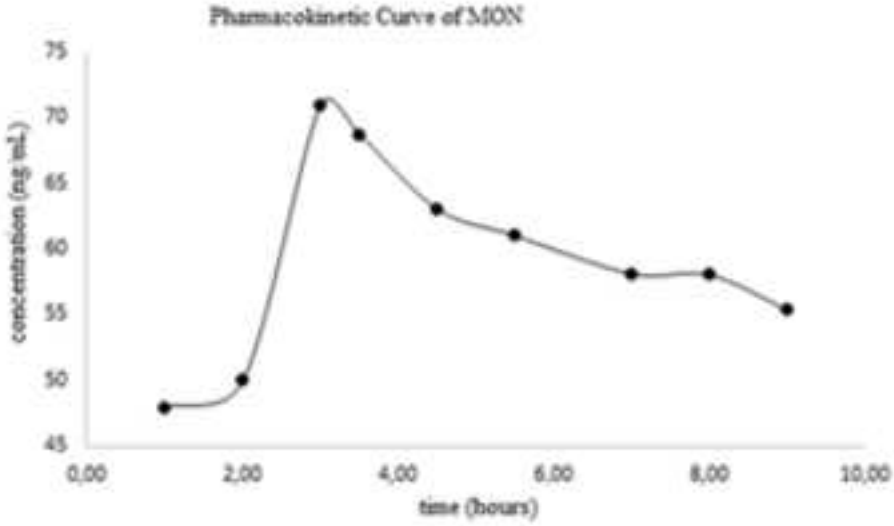


(a)



(b)

Table 1. Analytical and chromatographic parameters of the method

Parameters	FBX	MON
Concentration range [a] (ng mL^{-1})	0.3-10	5-100
Intercept± SD	- 54315 ±504.2	- 335712±768.7
Slope± SD	120867±93.022	49420±314.6
Correlation coefficient (r^2)	0.9947	0.9959
LOD (ng mL^{-1})	0.1	1.5
LOQ (ng mL^{-1})	0.3	5
Retention time	2.89	7.59
Tailing factor	1.213	0.973
HETP	35.959	11.403
Resolution	6.235	7.342
USP Width	0.177	0.285
Theoretical plates	4171.47	13154.88

[a] Average of six determinations; [b] $y=xC$ +b where C is the concentration in ng mL^{-1} and y is the peak area

Table 2. Precision and trueness of the method

	Added concentration (ng mL^{-1})	Found concentration (ng mL^{-1}) (Mean±SD[1])	Recovery (%)	RSD% recovery	RSD% intraday	RSD% interday
FBX	0.1	0.0906 ± 0.09	90.06	1.63	2.60	3.69
	5.00	4.926 ± 0.12	98.52	2.25	3.25	4.79
	10.00	9.765 ± 0.64	97.65	1.45	1.67	5.72
MON	5.00	4.673 ± 0.54	93.46	2.34	4.62	3.72
	50.00	46.32 ± 7.34	92.64	1.28	3.46	4.93
	100.00	98.16 ± 7.31	98.16	2.13	3.60	5.79

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[a] Average of six determinations; [b] ~~$y = xC$ +b where C is the concentration in ng mL^{-1} and y is the peak area~~ HETP: height equivalent to a theoretical plate

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	100.00	98.16 ± 7.31	98.16	2.13	3.60	5.79

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Table 2. Precision and trueness of the method

	Added concentration ($ng\ mL^{-1}$)	Found concentration ($ng\ mL^{-1}$) (Mean±SD[1])	Recovery (%)	RSD% recovery	RSD% intraday	RSD% interday
FBX	0.1	0.0906 ± 0.09	90.06	1.63	2.60	3.69
	5.00	4.926 ± 0.12	98.52	2.25	3.25	4.79
	10.00	9.765 ± 0.64	97.65	1.45	1.67	5.72
MON	5.00	4.673 ± 0.54	93.46	2.34	4.62	3.72
	50.00	46.32 ± 7.34	92.64	1.28	3.46	4.93
	100.00	98.16 ± 7.31	98.16	2.13	3.60	5.79

Table 3. Ruggedness of the method

Conditions		Analyte	Retention time (min)	Retention Factor (*k*)	Selectivity (α)
Mobile Phase solvents (acetonitrile:water)	60:40 (*v:v*)	FBX	2.9	6.502	6.398
		MON	7.6	2.145	1.649
	50:50 (*v:v*)	FBX	3.2	5.962	6.214
		MON	8.0	2.057	1.587
	70:30 (*v:v*)	FBX	2.2	4.658	6.057
		MON	6.9	1.957	1.324
Temperature	Room temperature	FBX	2.9	6.502	6.398
		MON	7.6	2.145	1.649
	35°C	FBX	3.0	6.324	5.940
		MON	7.8	2.144	1.625
	40°C	FBX	3.2	6.027	4.684
		MON	7.9	2.473	1.274
	45°C	FBX	3.4	5.172	4.254
		MON	8.0	1.571	1.174
Flow rate (change in 7 min)	from 0.5 to 1.5 mL min^{-1}	FBX	2.9	6.502	6.398
		MON	7.6	2.145	1.649
	from 0.8 to 1.8 mL min^{-1}	FBX	2.7	6.452	5.964
		MON	7.4	2.142	1.245
	from 0.3 to 1.2 mL min^{-1}	FBX	3.1	6.084	5.924
		MON	7.9	1.925	1.357

The gray boxes represent the optimized and validated conditions

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.

Isil Gazioglu: Conceptualization; S. Evrim Kepekci Tekkeli: Supervision, Reviewing, Investigation Methodology; Angela Tartaglia: Writing, Reviewing and Editing; Ceylin Aslan: Writing, Reviewing; Marcello Locatelli: Writing- Original draft preparation, Reviewing and Editing; Abuzar Kabir: Materials conceptualization and synthesis, Methodology, Writing- Reviewing and Editing

Simultaneous determination of febuxostat and montelukast in human plasma using fabric phase sorptive extraction and high performance liquid chromatography-~~fluorometric~~fluorimetric detection

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ABSTRACT

In the present work, a new sensitive and selective high-performance liquid chromatography–~~fluorometric~~-fluorimetric detection (HPLC-FLD) method was developed and validated to quantify ~~the~~ febuxostat (FBX) and montelukast (MON) in human plasma. The developed procedure was ~~then~~ successfully applied to a study aimed at evaluating the pharmacokinetic profiles of febuxostat and montelukast in human plasma. A sol-gel poly (caprolactone)-*block*-poly(dimethylsiloxane)-*block*-poly(caprolactone) (sol-gel PCAP-PDMS-PCAP) extraction sorbent coated fabric phase sorptive extraction membrane was used in the ~~plasma~~ extraction process. The ~~whole~~entire chromatographic analysis~~, carried out on a C18 stationary phase using~~ was performed with isocratic elution of the composition of the mobile phase (acetonitrile:-water ~~(~~, 60:40, *v:v*, 0.032% glacial acetic acid) ~~as the mobile phase in an isocratic elution with a variable~~ on the C18 column. The flow rate is varied during the analysis, particularly from 0.5 mL min^{-1} at the ~~beginning~~start and linearly increased ~~up~~ to ~~at~~ 1.5 mL min^{-1} in 7 min. The detection and quantification ~~was performed at~~ of the analytes was carried out by means of a fluorimetric detector at 320 nm and 350 nm as absorption ~~at 320 and 350 nm FBX~~ wavelengths and ~~MON, with~~at 380 and 400 nm as emission wavelengths ~~at 380 and 400 nm~~ for FBX and MON, respectively.

The calibration curves demonstrated linearity in the range 0.3-10 ng mL^{-1} and 5-100 ng mL^{-1} for FBX and MON, respectively, while the LOD and LOQ values were 0.1 and 0.3 ng mL^{-1} for FBX and 1.5 and 5 ng mL^{-1} for MON. ~~During the validation process, intraday~~Intraday and interday RSD% values were found lower than 5.79%. As reported, the method was ~~then~~ applied to ~~a~~ real plasma ~~sample~~samples obtained from a volunteer who was co-administered both the drugs ~~at the same time~~. Pharmacokinetic data reveal that the concentration of both the drugs reaches the plateau approximately at the same time, but exhibits an elimination phase at

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different rates. This study demonstrated the usefulness of the new method and its applicability in therapeutic drug monitoring (TDM).

Keywords: Febuxostat, Montelukast, Fabric Phase Sorptive Extraction, HPLC, Plasma ~~Analysis~~

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1. Introduction

Febuxostat (FBX), commercial name of 2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid, is a drug used nowadays in the treatment of gout that plays its function by decreasing the amount of uric acid in the blood. It represents the treatment of choice, especially if allopurinol is not adequate (or not usable) for this treatment [1]. The safety profile of this drug in terms of side effects are extensively studied and reported in the literature [2-6].

Montelukast (MON), commercial name of [R-(E)]-1-[[[[1-[3-[2-(7-chloro-2-quinolinyl) ethenyl]phenyl]-3-[2-(7-chlor-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl) phenyl]propyl]thio]methyl cyclopropane acetic acid sodium, is used as a modifier of leukotrienes widely used in case of bronchoconstriction and asthma [7]. This drug is often found in combination with others and for this reason its safety profile and its effectiveness can vary, making therapeutic drug monitoring essential in order to maintain its therapeutic effect. The chemical structures of FBX and MON are shown in **Figure 1**.

Figure 1: Chemical structures of FBX and MON

Following the increasingly frequent clinical practice of administering drug combinations [8], monitoring their simultaneous presence in biological matrix has become increasingly important in recent years and at the same time ~~nessesiates the development of~~it becomes crucial to develop new validated analytical methods in response to meet the increasing demand of clinical practice for the co-administered drugs.

To date, there are only two HPLC-based pharmacokinetic (PK) studies for FBX in rat plasma samples in the literature [9,10], and only a few methods for analysis in human plasma with LC-MS ~~/ /~~MS [11-14]. ~~Various~~Several HPLC methods for the determination of MON in plasma with UV detection or tandem mass spectrometry are reported in the literature [7,15-18].

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Only one work reports the simultaneous determination of ~~such~~these drugs in human plasma [19] and is based on high performance thin layer chromatography (HPTLC) combined with fluorescence detection. Furthermore, in the reported study, no real ~~sample was~~samples were analyzed, but only samples of an ~~"~~*ad-hoc*~~"~~ spiked biological matrix to verify the applicability of the procedure.

~~.~~It should also be noted that most of the reported methods ~~consider~~implemented the precipitation of proteins prior to the liquid-liquid extraction targetted to extract the analytes from the biological matrix.

Continuing our research on the characterization and application of fabric phase sorptive extraction (FPSE) on complex matrices [20-27], the objective of the current bioanalytical methodology is to synergistically exploit the high selectivity of the sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane in efficiently isolating FBX and MON from human plasma with detection and quantification in high performance liquid chromatography ~~hyphenated~~coupled with a highly sensitive and selective fluorimetric detector (HPLC-FLD).

The method was ~~then~~also applied for the first time to ~~a~~ real plasma ~~sample~~samples obtained from a volunteer who was co-administered both the drugs ~~at the same time.~~ The entire process was approved by the local Ethical Committee and ~~Pharmacokinetic~~pharmacokinetic data reveal that the concentration of both drugs reaches the plateau approximately at the same time, but exhibits an elimination phase at different rates. This study demonstrated the usefulness of the new method and its applicability in therapeutic drug monitoring (TDM).

2. Experimental

~~2.1.~~ ~~.~~2.1 *Chemicals and Reagents*

The sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane was synthesized at the Department of Chemistry and Biochemistry of Florida International University (Miami,

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Florida, USA). In the FPSE membrane synthesis process, methyl trimethoxysilane (MTMS), acetone, trifluoroacetic acid (TFA) and dichloromethane were purchased from Sigma-Aldrich (St. Louis, MO, USA), while PCAP-PDMS-PCAP was purchased from Gelest Inc. (Morrisville, PA, USA). Sodium hydroxide, and hydrochloric acid was supplied by Thermo Fisher Scientific (Milwaukee, WI, USA). Unbleached muslin (100% cotton) membrane substrates were purchased from Jo-Ann Fabric (Miami, FL, USA).

The chemical standards of FBX and MON (analytical grade, purity ≥99%) were purchased from Shanghai Yingxuan Pharmaceutical Science & Technology (China), while the drugs for human use Adenuric® (80 mg of FBX) and Singulair® (5 mg MON), were bought in a local pharmacy (Istanbul, Turkey). Acetonitrile, methanol, and glacial acetic acid (HPLC grade, Merck, Darmstadt, Germany) were used without further purification steps, and the water was purified using a Human Ultra Water Purification System (Japan).

~~2.2.~~ 2.2 *Preparation of standard solutions*

The stock solutions of the two analytes FBX and MON at a concentration of 0.1 mg mL^{-1} were obtained by solubilizing the chemical standards in methanol and subsequently diluted to obtain working solutions from 0.1 to 10 ng mL^{-1} (FBX) and 5 to 100 ng mL^{-1} (MON). The stock and working solutions were stored at 4 °C and proved stable for the duration of the study.

~~2.3.~~ 2.3 *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, a system of Barnstead NANOPure Diamond deionized

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water (Model D11911, Dubuque, IA). 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 ~~was used for the SEM analysis.~~ Philips XL scan 30 equipped with an EDAX detector was used for the SEM analysis.

The HPLC ~~analyzes~~analyses were performed using a Shimadzu (Japan) LC 20 liquid chromatograph equipped with LC-20AT pump, SIL-20A HT autosampler, RF-20A fluorimetric detector (set to wavelengths of 320 and 350 nm for excitation, and 380 and 400 nm for the emission of FBX and MON, respectively). The chromatographic column was thermostated at room temperature (25°C±1°C) using a CTO-10AS VP oven and the resolution of the two analytes was obtained using a Phenomenex Luna C18 (ODS) column (4.6 mm x 125 mm, 5 µm).

The entire chromatographic analysis~~, carried out using~~ was performed with isocratic elution of the composition of the mobile phase (acetonitrile: water ~~(~~, 60:40, *v:v*, 0.032% ~~of~~ glacial acetic acid) ~~as the mobile phase in an isocratic elution (in terms of percentage composition of the phase) with a varying~~on the C18 column. The flow rate is varied during the analysis, particularly from 0.5 mL min^{-1} ~~in~~at the ~~beginning~~start and linearly increased to 1.5 mL min^{-1} in 7 min. Under these conditions, the entire chromatographic run took 13 min., allowing the resolution of FBX and MON without any matrix interference. A representative chromatogram obtained from plasma spiked with 50 ng/mL of the target analytes is shown in **Figure 2**.

~~**Figure 2: (a)** Chromatogram of blank plasma, **(b)** The chromatograms obtained from plasma spiked with 5 ng mL^{-1} for FBX and 50 ng mL^{-1} for MON, **(c)** The chromatograms obtained from the healthy volunteer after 3 h of drugs administration~~

~~2.4.~~ 2.4 *Sample preparation and extraction*

The plasma samples obtained from blood draws from the peripheral veins of the volunteers (5 mL) used in the present study, provided by the blood bank of the Bezmialem Vakif University

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Hospital of the Istanbul Faculty of Medicine (Turkey, ~~ethics committee~~Ethical Committee approval, No. 15/10) were stored in test tubes containing disodium EDTA and centrifuged at 4500~~ × ×~~*g* for 10 min. and kept at -20°C until analysis.

For the extraction process of FBX and MON, the 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: acetonitrile (40:60, *v:v*) for 5 minutes; *iii)* the FPSE membranes were rinsed in deionized water; *iv)* 20 µL of plasma were diluted with 280 µL of isotonic solution (0.9% sodium chloride); *v)* 100 µL portions of standard solutions at different concentrations (for the calibration curve and the QC quality control samples) were added for each drug; *vi)* the extraction was carried out under magnetic stirring at 200 rpm at room temperature~~;~~ for 30 min, *vii)* the ~~retro~~back-extraction of the analytes from the FPSE membranes was carried out by means of ~~a small volume~~100 µL of ~~organic solvent~~methanol for 10 min; *viii)* finally 10 µL of the extract were injected into the chromatographic system.

~~2.5.~~ 2.5 *Preparation of sol-gel PCAP-PDMS-PCAP coated fabric phase sorptive extraction membrane*

In the preparation of the sol solution for the creation of the PCAP-PDMS-PCAP sol-gel coating on 100% cotton fabric substrate, poly(caprolactone)-*block*-poly(dimethylsiloxane)-*block*-poly(caprolactone) polymer was mixed under vortex for 3 min., methyl trimethoxysilane, methylene chloride, trifluoroacetic acid and deionized water in a 50 mL reaction vessel with a molar ratio of 0.07:1:94:2.3:0.75:3, respectively, to ensure a homogeneous mixture. In the solution thus obtained, sonicated for 10 min., a cellulose fabric (15 ~~cm~~ x 45 cm) ~~is~~was immersed to ~~thicken~~create the sol-gel coating through a process carried out at room temperature for 8 h. At the end, the sol-gel sorbent membrane was removed, dried in air for 1 h, and subjected to heat treatment at 50°C for 24 h under a continuous flow of nitrogen gas. The obtained FPSE

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membrane was then washed with a 50:50 (*v:v*) mixture of methylene chloride and methanol under sonication for 1 h and treated again at 50°C for 12 h. The FPSE membrane coated with sol-gel PCAP-PDMS-PCAP sorbent was then stored in an airtight container until its use for the characterization and application in plasma ~~sample~~samples preparation.

~~*2.6. Validation of the*~~*2.6 FPSE-HPLC-FLD method validation*

The method herein reported ~~here has been~~was validated according to the International Conference on Harmonization (ICH) and CDER Guidelines [28,29] for analytical parameters such as: linearity, precision and trueness (both intraday and interday), LOD, LOQ, ~~robustness~~ruggedness and stability.

3. Results and discussion

~~*3.1.*~~ *3.1 Selection of the FPSE sorbent chemistry*

Compared to the conventional (micro)-extraction procedures based on sorbents, in the case of FPSE, a functionalized three-dimensional network is created and suitable for maximizing the retention of the analytes on *(a)* a fabric substrate that not only hosts the network of sol--gel sorbent, but also contributes to the overall selectivity of the FPSE membrane via its intrinsic hydrophilic/hydrophobic attribute; *(b)* a sol-gel precursor which acts as a bridge between the fabric substrate and the sol-gel sorbent network; *(c)* an organic/inorganic polymer that provides the primary interaction between the analyte(s) and the membrane via its diversified functional groups.

The major advantage of this technique lies in the fact that it allows to ~~reconcile~~couple the principles of exhaustive extraction (typical of solid phase extraction, SPE) with the principles of equilibrium extraction (typical of solid phase microextraction, SPME).

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In the present work, given that the two analytes considered in the current study show a large difference in polarity (mid-polar ~~mean~~ FBX, and non-polar MON), the greatest analytical challenge was the creation and selection of a FPSE phase ~~capable of extracting~~able to extract these compounds with maximum efficiency.

In the present work, the ~~objective~~target was achieved through the use of an FPSE membrane containing sol-gel poly (caprolactone)-*block*-poly(dimethylsiloxane)-*block*-poly(caprolactone). In fact, in this phase, poly(caprolactone) is a polar, biocompatible and biodegradable polymer. Poly(dimethylsiloxane) is a non-polar and uniquely biocompatible polymer. The simultaneous presence of both functionalities made the polymer an ideal extraction sorbent for the ~~current~~ analytical challenge encountered in the current study.

In the polymer synthesis phase, methyl trimethoxysilane was selected as a sol-gel precursor for its terminal methyl group which can exert an additional interaction of the London dispersion type towards the analytes during the FPSE process, but also because in the sol-gel synthesis, it represents one of the best available linkers.

The selected substrate (cotton muslin, 100% cellulose) ~~derives from the fact that it~~ shows an intrinsic hydrophilic character, essential ~~for obtaining~~to obtain a seamless mass transfer of the analytes from thec aqueous sample matrix ~~in the immediate vicinity of~~to the sol-gel sorbent ~~such as~~coated FPSE membrane via the boundary layer in order to maximize the interaction ~~of~~between the target analytes with the sol-gel PCAP-PDMS-PCAP through different intermolecular interactions, leading to rapid and effective extraction.

~~3.2.~~ ~~3.2~~ *Characterization of FPSE membrane*

The FPSE membranes were characterized by Fourier transform infrared spectroscopy analysis techniques and by scanning electron microscopy techniques~~, based on what reported below.~~

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~~*3.2.1. Scanning Electron Microscopy*~~

. Scanning electron microscopy (SEM) allows elucidating the surface morphology of the sol-gel sorbent coating by revealing information about the uniformity of the coating. Given the principles on which the technique is based, the possibility of having a substrate available with distinct pores even after the sol-gel coating process is ~~of~~ fundamental ~~importance.~~

. **Figure 3** shows the SEM images of **(a)** substrate of uncoated cellulose fabric at 100x magnification respectively; **(b)** FPSE membrane coated with PCAP-PDMS-PCAP sol-gel sorbent at 100x magnification; and **(c)** PCAP-PDMS-PCAP sol-gel coated FPSE membrane at ~~5000~~5000x magnification.

Following ~~this analysis, it is~~these analyses, was observed that the FPSE membrane reveals the presence of unique pores of the cellulose fabric substrate which are preserved even after coating. At the same time, ~~it is~~was observed that the sol-gel sorbent coating is uniformly distributed on the substrate and that any differences between the uncoated and coated fabric are difficult to ~~recognize~~distinguish. A further magnification (**Figure 3c**) allows to reveal how the absorbent is distributed uniformly around the single cellulose microfibril ~~of cellulose~~ with the consequent presence of a rough surface of the sol-gel coating.

~~*3.2.2. Fourier Transform Infrared Spectroscopy*~~

Figure 3. SEM images and FT-IR spectra of the FPSE membranes used in the present validated method: **(a)** SEM of uncoated cellulose fabric at 100x magnifications; **(b)** SEM of sol-gel PCAP-PDMS-PCAP coated FPSE membrane at 100x magnifications; **(c)** SEM of sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane at 5000x magnifications; **(d)** FT-IR spectra of Methyltrimethoxysilane; **(e)** FT-IR spectra of original poly(caprolactone)-*b*-poly(dimethylsiloxane)-*b*-poly(caprolactone) polymer; **(f)** FT-IR spectra of sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane.

Based on the FT-IR analysis reported in **Figure ~~4 (ac~~3 (def)** and referring to the main constituent elements of the FPSE membrane, methyltrimeoxysilane, poly(caprolactone)-*block*-

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--poly (dimethylsiloxane)-*block*-poly (caprolactone) and the coated FPSE membranes coated with sol-gel PCAP-PDMS-PCAP sorbent, it is possible to observe in **(~~a~~d)** the characteristic bands at 1265 cm^{-1} and 788 cm^{-1} ~~relating~~related to the vibration of CH_3 group of the precursor [30], while the signals at 1188 cm^{-1} and 1076 cm^{-1} are attributable to the CO stretching of Si-O-CH_3. The signals at 2840 cm^{-1} and 1465 cm^{-1} can be assigned to the C-H stretching and bending vibration of Si-O-CH_3, respectively [31]. Noteworthy bands obtained from the polymer poly(caprolactone)-*block*-poly(dimethylsiloxane)-*block*-poly (caprolactone) include the stretching of the carbonyl at 1722 cm^{-1}, the asymmetric stretching of CH_2 at 2949 cm^{-1}, the symmetrical stretch of CH_2 at 2865 cm^{-1}, CO stretching at 1292 cm^{-1}. There is also a strong signal at 795 cm^{-1} which could be attributed to CH_3 rocking originating from the PDMS block of the polymer, while that at 1246 cm^{-1} to the symmetrical folding of Si(CH_3), a characteristic feature of the PDMS block [32]. Many bands appeared simultaneously in both MTMS and poly(caprolactone)-*block*-poly (dimethylsiloxane)-*block*-poly (caprolactone) and the sol-gel PCAP-PDMS-PCAP sorbent –coated FPSE membrane strongly suggests successful integration of both the components in the FPSE membrane.

~~3.3.~~ -3.3 *Optimization of FPSE procedure*

Three different FPSE membrane chemistries were primarily evaluated: Sol-gel MTMS/PheTES/PDMS, Substrate: Thick Polyester, Batch: 092519; Sol-gel MTMS/PheTES/PDMS, Substrate: Cellulose, Batch: 091619_1 and 091619_2; and sol-gel PCAP-DMS-CAP Substrate: Polyester, Batch: 020819_1. According to the recovery results, sol-gel PCAP-DMS-CAP membrane shows better performances and was selected for further tests for the analyte extraction. The other membranes demonstrated low recovery of the target analytes. To obtain the best performance from the FPSE extraction procedure, it was necessary to optimize various FPSE conditions, such as: *i)* extraction times*; ii)* back-extraction time; *iii)*

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type of back-extraction/elution solvent; *iv)* back-extraction solvent volume. The various conditions tested are: from 5 to 60 min. for extraction and back-extraction; acetonitrile, acetonitrile: methanol (40:60, *v:v*), acetonitrile: water (60:40, *v:v*), methanol, methanol: water (60:40, *v:v*) as back-extraction solvents with different volumes.

Furthermore, as reported in the literature [33,34], in order to reduce the process times, the extraction by vortexing at 200 rpm, room temperature for 30 min 20 µL of plasma diluted with 280 µL of physiological (0.9% NaCI) solution was also tested. Following the various conditions tested, the optimum procedure was found to be: *i)* the membranes were cut into squares of 1 cm^2 of surface; *ii)* the FPSE membrane was cleaned and activated by methanol: acetonitrile (40:60, *v:v*) for 5 minutes; *iii)* the FPSE membranes were rinsed in deionized water; *iv)* 20 µL of plasma were diluted with 280 µL of isotonic solution (0.9% sodium chloride); *v)* 100 µL portions of standard solutions at different concentrations (for the calibration curve and the QC quality control samples) were added for each drug; *vi)* the extraction was carried out under magnetic stirring at 200 rpm at room temperature for 30 min; *vii)* the ~~retro~~back-extraction of the analytes from the membranes was carried out by means of ~~a small volume~~100 µL of ~~organic solvent~~methanol for 10 min; *viii)* finally 10 µL of the extract were injected into the chromatographic system.

~~*3.4. Chromatographic process*~~

3.4 Optimization of the chromatographic separation

The chromatographic analysis with the optimized conditions previously reported allowed the complete resolution of the analytes in 13 min. reaching a high selectivity as the presence of matrix interferers is not observed. During the method development exercises, different HPLC parameters and mobile phase compositions were evaluated. For the separation different columns like Spherisorb ODS1 C18 column (4.6 mm×150 mm, 5 µm) and Phenomenex Luna

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C18(ODS) column (4.6 mm x 150 mm, 5 µm), different mobile phase compositions (acetonitrile:water, 60:40, *v:v*, 0.032% glacial acetic acid and methanol:water, 70:30, *v:v*, 0.032% glacial acetic acid), different temperatures (25°C, 35°C, 40°C, and 45°C) and different flow rates ramps (from 0.5 to 1.5 in 7 min, from 0.8 to 1.8, and from 0.3 to 1.2 mL min^{-1} over the same time) were evaluated. Best separation conditions were achieved with Phenomenex Luna C18(ODS) column, under isocratic elution of the composition of the mobile phase (acetonitrile:water, 60:40, *v:v*, 0.032% glacial acetic acid), temperature 25°C (±1°C), and the flow rate is varied during the analysis, particularly from 0.5 mL min^{-1} at the start and linearly increased to 1.5 mL min^{-1} in 7 min. These conditions allow to obtain the better signal-to-noise (S/N) values, peak shapes and suitable retention times for routine analyses in clinical field, than other tested conditions.

~~**Figure 5 (ac**~~The selectivity, tested using blank plasma samples shows that the method was selective for the FBX and MON and the retention times of drugs are comparable to those obtained for real samples and no interfering peaks were detected during the analysis. **Figure 2 (a-c)** shows respectively the chromatographic profile of the blank matrix, and the chromatograms obtained from the analysis of blank plasma added with 5 ng mL^{-1} for FBX and 50 ng mL^{-1} for MON, and the chromatograms obtained from the healthy volunteer after 3 hours of drug administration.

~~*3.4.1. Preparing calibration curve and calculation of method sensitivity*~~

3.5 Analytical figure of merits

The validated calibration curves of the method herein reported were obtained by least squares linear regression analysis by plotting the peak areas of the analytes *vs.* concentration levels. The mean equation of the calibration curve (n = 6) obtained on seven concentration levels were: y = 120867x- 54315 (correlation coefficient = 0.9947) for FBX, and y = 49420x-

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335712 (correlation coefficient = 0.9959) for MON. All the parameters related to the analytical method performance are shown in **Table 1**.

~~*3.4.2 Trueness, precision and recovery*~~**Table 1.** Analytical and chromatographic parameters of the method

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Both intraday and interday precision and trueness were evaluated by analyzing samples for quality control (QC) at three different concentrations (0.3, 5, 10 ng mL^{-1} for FBX and 5, 50 and 100 ng mL^{-1} for MON) and classified as low, medium and high concentration. The analyses were carried out in sixfold (n = 6) in the blank matrix and in the aqueous sample. Final recoveries of FBX and MON from the biological matrix were evaluated by extraction of spiked plasma samples and compared with the peak areas obtained from the same quantities of unextracted aqueous solutions of the drugs. The mean absolute recovery observed stood at values of 89.2 and 92.3%, while the relative recoveries were 95.9 and 95.5% for FBX and MON, respectively.

Each test was performed both on the same day for intraday variability assessment and on six different days for interday precision (intermediate precision) and trueness. The RSD% values of the intraday and interday analyzes were all less than 5.79%. All these results have been summarized in **Table 2**.

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~~*3.4.3. Ruggedness*~~

Table 2. Precision and trueness of the FPSE-HPLC-FLD method

The ~~robustness~~ruggedness of the method, understood as the reproducibility of the method following small but deliberate variations in method parameters, was evaluated at different flow

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values, temperatures of the column furnace and different ratios of the mobile phase content. In these tests, the percentage composition of the mobile phase was varied from 60:40 to 50:50 and ~~25:75.~~70:30 (*v:v*). The temperature of the column was changed from ~~40ºC~~25°C to 35ºC, 40°C, and 45ºC, while the flow from 0.5 to 1.5 in 7 min was ranged from 0.8 to 1.8 and from 0.3 to 1.2 mL min^{-1} over the same time. The variations studied showed that the chromatographic conditions had no effect on the peak area and on the analytical performances of the procedure, as reported in **Table 3**.

~~*Stability study*~~**Table 3.** Ruggedness of the FPSE-HPLC-DAD method

~~*3.5.*~~

During the validation process of the method, the stability of the analytes and any effects of the storage conditions on them were also evaluated. These studies were evaluated considering blank plasma samples added to the 3 QC levels and subjected to four freeze-thaw cycles before analysis, as well as monitoring stability at room temperature for 24 h, and at –20°C for 2 weeks. The stock solutions of FBX and MON were stable for at least 30 days when stored at -20ºC. After 30 days, no change in the chromatographic profiles of the analytes was observed. The solutions were stable for 4 days if the samples were kept at 4°C using a sample cooler, 1 day at room temperature.

~~*3.6.*~~ ~~*3.6*~~ *Application ~~of the composite method~~ to real plasma analysis*

The validated method was then applied to the analysis of real samples to evaluate the pharmacokinetic profile (PK) on a healthy 24-year-old volunteer who was administered a single oral dose of 80 mg FBX and 15 mg MON. 5 mL of venous blood samples were collected prior to drug administration (baseline) and 1, 2, 3, 4, 6, 8, 10, and 12 h after the first day based on the pharmacokinetics of both drugs. The blood samples were then processed according to the

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method described above. Specifically, **Figure 2c** shows the chromatogram of the plasma sample obtained 3 h after administration. Since matrix stability was evaluated, the samples were stored at –20°C until analysis. The pharmacokinetic parameters were calculated using the analysis performed with this method to derive the area values under the plasma concentration-time curves (AUC_{0-12}, $AUC_{0-\infty}$) using the TOPFIT 2.0 pharmacodynamic and pharmacokinetic data analysis system. The plasma concentration-time curves of FBX and MON following oral administration of a single dose of 80 mg FBX and 15 mg MON are shown in **Figure ~~5~~4.**

Figure 4. (a) Pharmacokinetic curve of FBX, **(b)** Pharmacokinetic curve of MON

Conclusions

The method validated in the present work is able to simultaneously determine FBX and MON drugs widely used ~~respectively~~ for the treatment of gout and asthma, respectively in plasma matrix. The method represents an excellent procedure for clinical applications aimed at therapeutic drug monitoring since, following the increase in clinical practice of using drug combinations, it is evident that obtaining the therapeutic effect, reducing/eliminating adverse effects, and above all the therapeutic window within well-defined concentration ranges, is of fundamental importance especially when dealing with drugs that may have drug interactions among each other.

This method allows overall cost reduction, reduces analysis time, provides a simple analysis for simultaneous quantification in plasma samples with a superior selectivity and sensitivity to meet modern day clinical needs. In the literature, there are some methods for the analysis of these substances separately, however, there is only one method for the simultaneous ~~dosing for~~monitoring of both the drugs in HPTLC. The advantages of this procedure mainly depend on the pretreatment method performed by the FPSE technique. This extraction approach

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is a relatively new and more efficient, environmentally friendly, and simpler technique than conventional extraction procedures such as liquid-liquid and solid-phase extraction techniques. After this simple pretreatment, reverse phase chromatography and fluorometric detection provided high sensitivity and selective analysis in the plasma samples. From this study, it is clear that the presented method is a valid procedure for plasma analysis in patients undergoing such combination treatment.

Acknowlegments

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Declaration of Competing Interest

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

References

[1] H.R. Manasse, W.A. Zellmer, American Society of Health-System Pharmacists, Pharmacotherapy 21 (2001) 123-125.

[2] M.A. Becker, H.R. Schumacher, L.R. Espinoza, A.F. Wells, P. MacDonald, E. Lloyd, C. Lademacher, The urate-lowering efficacy and safety of febuxostat in the treatment of the hyperuricemia of gout: the CONFIRMS trial, Arthritis Res Ther 12(2) (2010).) article R63, 1-12.

[3] E. Roddy, M. Doherty, Epidemiology of gout, Arthritis Res Ther 12(6) (2010).) article 223, 1-11.

[4] I.S. Mackenzie, I. Ford, G. Nuki, J. Hallas, C.J. Hawkey, J. Webster, S.H. Ralston, M. Walters, M. Robertson, R. De Caterina, E. Findlay, F. Perez-Ruiz, J.J.V. McMurray, T.M. MacDonald, F.S. Grp, Long-term cardiovascular safety of febuxostat compared with allopurinol in patients with gout (FAST): a multicentre, prospective, randomised, open-label, non-inferiority trial, Lancet 396 (2020) 1745-1757.

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[5] F.P. Ruiz, P. Richette, A.G. Stack, R.K. Gurunath, M.J.G. de Yebenes, L. Carmona, Failure to reach uric acid target of < 0.36 mmol/L in hyperuricaemia of gout is associated with elevated total and cardiovascular mortality, Rmd Open 5 (2019~~).~~) e001015, 1-10.

[6] W.B. White, K.G. Saag, M.A. Becker, J.S. Borer, P.B. Gorelick, A. Whelton, B. Hunt, M. Castillo, L. Gunawardhana, C. Investigators, Cardiovascular Safety of Febuxostat or Allopurinol in Patients with Gout, New Engl J Med 378 (2018) 1200-1210.

[7] S. Al-Rawithi, S. Al-Gazlan, W. Al-Ahmadi, I.A. Alshowaier, A. Yusuf, D.A. Raines, Expedient liquid chromatographic method with fluorescence detection for montelukast sodium in micro-samples of plasma, J Chromatogr B 754 (2001) 527-531.

[8] C. Celia, L. Di Marzio, M. Locatelli, P. Ramundo, F. D'Ambrosio, A. Tartaglia, Current trends in simultaneous determination of co-administered drugs, Separations 7(2) (2020), article 29, 1-18.

[9] B.B. Gabani, N.K. Saini, R.K. Jairam, P. Shrinivas, R.K. Trivedi, N.R. Srinivas, R. Mullangi, Simultaneous determination of colchicine and febuxostat in rat plasma: Application in a rat pharmacokinetic study, Biomed Chromatogr 34(11) (2020~~).~~) article e4939, 1-11.

[10] D.G. Han, K.S. Kim, S.W. Seo, Y.M. Baek, Y. Jung, D.D. Kim, I.S. Yoon, A sensitive HPLC-FL method to simultaneously determine febuxostat and diclofenac in rat plasma: assessment of metabolic drug interactionsin vitroandin vivo, Anal Methods ~~-Uk~~ 12 (2020) 2166-2175.

[11] Y.C. Xu, J.L. Chen, D.D. Yang, Y. Hu, X.H. Hu, B. Jiang, Z.R. Ruan, H.G. Lou, Development of LC-MS/MS determination method and backpropagation artificial neural networks pharmacokinetic model of febuxostat in healthy subjects, J Clin Pharm Ther 46 (2021) 333-342.

[12] D. Tandel, P. Shah, K. Patel, V. Thakkar, K. Patel, T. Gandhi, Salting-Out Assisted Liquid-Liquid Extraction for Quantification of Febuxostat in Plasma Using RP-HPLC and Its Pharmacokinetic Application, J Chromatogr Sci 54 (2016) 1827-1833.

[13] B. Kamel, K.M. Williams, G.G. Graham, R.L.G. Norris, S.L. Stocker, J.E. Carland, K.D. Pile, R.O. Day, Determination of febuxostat in human plasma by high performance liquid chromatography (HPLC) with fluorescence-detection, ~~Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 1126 (2019).~~J Chromatogr B 1126-1127 (2019) article 121764, 1-8.

[14] F.A. El-Yazbi, O.A. Amin, E.I. El-Kimary, E.F. Khamis, S.E. Younis, M.A.W. Elkhatib, A.F. El-Yazbi, A novel HPLC-DAD method for simultaneous determination of febuxostat and diclofenac in biological samples: pharmacokinetic outcomes, Bioanalysis 11 (2019) 41-54.

[15] G.A. Smith, C.M. Rawls, R.L. Kunka, An automated method for the determination of montelukast in human plasma using dual-column HPLC analysis and peak height summation of the parent compound and its photodegradation product, Pharm Res 21 (2004) 1539-1544.

[16] N.S. Rashed, Z.A. Nasr, Simultaneous Determination of Levocetirizine Dihydrochloride and Montelukast Sodium in Human Plasma by LC-MS/MS: Development, Validation, and Application to a Human Pharmacokinetic Study, Acta Chromatogr 31 (2019) 194-200.

[17] A.K. Shakya, T.A. Arafat, N.M. Hakooz, A.N. Abuawwad, H. Al-Hroub, M. Melhim, High-Performance Liquid Chromatographic Determination of Montelukast Sodium in Human Plasma: Application to Bioequivalence Study, Acta Chromatogr 26 (2014) 457-472.

[18] R. Muppavarapu, S. Guttikar, M. Rajappan, K. Kamarajan, R. Mullangi, Sensitive LC-MS/MS-ESI method for simultaneous determination of montelukast and fexofenadine in

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human plasma: application to a bioequivalence study, Biomed Chromatogr 28 (2014) 1048-1056.
[19] N.M. Hosny, N.N. Atia, S.M. El-Gizawy, D.M. Badary, M.S. Hareedy, Innovative HPTLC method with fluorescence detection for assessment of febuxostat-montelukast combination and study of their protective effects against gouty arthritis, Analyst 143 (2018) 4366-4378.
[20] A. Kabir, K.G. Furton, N. Tinari, L. Grossi, D. Innosa, D. Macerola, A. Tartaglia, V. Di Donato, C. D'Ovidio, M. Locatelli, Fabric Phase Sorptive Extraction-High Performance Liquid Chromatography-Photo Diode Array Detection Method for Simultaneous Monitoring of Three Inflammatory Bowel Disease Treatment Drugs in Whole Blood, Plasma and Urine, ~~Journal Of Chromatography~~J Chromatogr B 1084 (2018) 53-63.
[21] M. Locatelli, A. Tartaglia, F. D'Ambrosio, P. Ramundo, H.I. Ulusoy, K.G. Furton, A. Kabir, Biofluid sampler: A new gateway for mail-in-analysis of whole blood samples, ~~Journal Of Chromatography~~J Chromatogr B 1143 (2020) article 122055, 1-7.
[22] A. Tartaglia, A. Kabir, F. D'Ambrosio, P. Ramundo, S. Ulusoy, H.I. Ulusoy, G.M. Merone, F. Savini, C. D'Ovidio, U. De Grazia, K.G. Furton, M. Locatelli, Fast off-line FPSE-HPLC-PDA determination of six NSAIDs in saliva samples, ~~Journal Of Chromatography~~J Chromatogr B 1144 (2020) article 122082, 1-9.
[23] E. Agadellis, A. Tartaglia, M. Locatelli, A. Kabir, K.G. Furton, V. Samanidou, Mixed-mode fabric phase sorptive extraction of multiple tetracycline residues from milk samples prior to high performance liquid chromatography-ultraviolet analysis, ~~Microchemical Journal,~~Microchem J 159 (2020) article 105437, 1-10.
[24] M. Locatelli, A. Tartaglia, H.I. Ulusoy, S. Ulusoy, F. Savini, S. Rossi, F. Santavenere, G.M. Merone, E. Bassotti, C. D'Ovidio, E. Rosato, K. Furton, A. Kabir, Fabric phase sorptive membrane array as non-invasive in vivo sampling device for human exposure to different compounds, ~~Analytical Chemistry~~Anal Chem 93(4) (2021) 1957-1961.
[25] M. Locatelli, N. Tinari, A. Grassadonia, A. Tartaglia, D. Macerola, S. Piccolantonio, E. Sperandio, C. D'Ovidio, S. Carradori, H.I. Ulusoy, K.G. Furton, A. Kabir, FPSE-HPLC-DAD method for the quantification of anticancer drugs in human whole blood, plasma and urine, ~~Journal Of Chromatography~~J Chromatogr B 1095 (2018) 204-213.
[26] I. Gazioglu, A. Kabir, O.S. Zengin, E.K. Tekkeli, K.G. Furton, A. Tartaglia, M. Locatelli, Development of sol-gel phenyl/methyl/poly (dimethylsiloxane) sorbent coating for fabric phase sorptive extraction and its application in monitoring human exposure to selected polycyclic aromatic hydrocarbons using high performance liquid chromatography-fluorescence detection, ~~Journal Of Chromatography~~J Chromatogr B 1163 (2021) article 112520, 1-8.
[27] I. Gazioglu, O. Sultan Zengin, A. Tartaglia, M. Locatelli, K.G. Furton, A. Kabir, Determination of polycyclic aromatic hydrocarbons (PAHs) in nutritional supplements by fabric phase sorptive extraction (FPSE) with high-performance liquid chromatography (HPLC) with fluorescence detection, ~~Analytical Letters~~Anal Lett 54(10) (2021) 1683-1696.
[28] CDER e CVM, Bioanalytical Method Validation-Guidance for Industry, Food and Drug Administration, May 2018. https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf Last access: 11/11/2021.
[29] International Conference on Harmonization of Technical Requirements for registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline (2005) Validation of Analytical Procedures: Text and Methodology Q2(R1), Geneva, 2005. https://database.ich.org/sites/default/files/Q2_R1__Guideline.pdf Last access: 11/11/2021.

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[30] V. Samanidou, L.-D. Galanopoulos, A. Kabir, K.G. Furton, Fast extraction of amphenicols residues from raw milk using novel fabric phase sorptive extraction followed by high-performance liquid chromatography-diode array detection, ~~Analytica Chimica~~Anal Chim Acta 855 (2015) 41-50. ~~http://dx.doi.org/10.1016/j.aca.2014.11.036.~~

[31] B. Xu, J.J. Ge, Z.S. Cai, One-Step Processing to Fabricate Highly Transparent Superhydrophobic Surface via Granuliform Silica Aerogels, ~~Advanced Materials Research~~Adv Mat Res 936 (2014) 1042-1046. ~~10.4028/www.scientific.net/AMR.936.1042.~~

[32] C. Gordin, M. Rusu, C. Delaite, S. Salhi, T. Elzein, M. Brogly, Crystalinity Behaviour in Poly(epsilon-caprolactone)-b-Poly(dimethylsiloxane) Diblock and Triblock Copolymers through FTIR and DSC, Mater~~.~~ Plast~~.~~ 46 (2009) 37-42.

[33] A. Kabir, M. Locatelli, H.I. Ulusoy, Recent Trends in Microextraction Techniques Employed in Analytical and Bioanalytical Sample Preparation, Separations 4(4) (2017~~).~~) article 36, 1-15.

[34] S. Gülle, H.I. Ulusoy, A. Kabir, A. Tartaglia, K.G. Furton, M. Locatelli, V.F. Samanidou, Application of fabric phase sorptive extraction-high performance liquid chromatography-photodiode array detection method for trace determination of Methylparaben, Propylparaben and Butylparaben in Cosmetic and Environmental Samples, ~~Analytical~~Anal Methods 11 (2019) 6136-6145.

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Figure and Tables Captions

Figure 1: Chemical structures of FBX and MON

~~**Figure 2: (a)** Chromatogram of blank plasma, **(b)** The chromatograms obtained from plasma spiked with 5 ng mL^{-1} for FBX and 50 ng mL^{-1} for MON, **(c)** The chromatograms obtained from the healthy volunteer after 3 h of drugs administration~~

Figure 2: (a) Chromatogram of blank plasma, **(b)** The chromatograms obtained from plasma spiked with 5 ng mL^{-1} for FBX and 50 ng mL^{-1} for MON, **(c)** The chromatograms obtained from the healthy volunteer after 3 h of drugs administration

Figure 3. ~~Scanning electron microscopy~~SEM images ~~of **(a)**~~and FT-IR spectra of the FPSE membranes used in the present validated method: **(a)** SEM of uncoated cellulose fabric at 100x magnifications; **(b)** SEM of sol-gel PCAP-PDMS-PCAP coated FPSE membrane at 100x magnifications; **(c)** SEM of sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane at ~~5,000x~~5000x magnifications~~.~~

~~**Figure 4.**~~; **(d)** FT-IR spectra of ~~**(a)**~~ Methyltrimethoxysilane; ~~**(b)** pristine~~**c)** FT-IR spectra of original poly(caprolactone)-*b*-poly(dimethylsiloxane)-*b*-poly(caprolactone) polymer; ~~**(c)**~~**f)** FT-IR spectra of sol-gel PCAP-PDMS-PCAP sorbent coated FPSE membrane.

Figure ~~5~~4. (a) Pharmacokinetic curve of FBX, (**b)** Pharmacokinetic curve of MON

Table 1. Analytical and chromatographic parameters of the FPSE-HPLC-FLD method
Table 2. Precision and trueness of the FPSE-HPLC-FLD method
Table 3. Ruggedness of the FPSE-HPLC-FLD method

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