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The Extraction of Biological Matrices by FPSE – GC – MS Technique

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Abstract

During the pandemic emergency in 2020, favipiravir, a pyrazine analog, is proposed as a providential antiviral agent against the COVID-19 infection. Fabric phase sorptive extraction (FPSE) and gas chromatography-mass spectrometry (GC-MS) have been developed and used for the first time to identify favipiravir (FAV) in pharmaceutical, forensic, and biological samples (human plasma, blood, and urine). FAV is extracted using FPSE, its derivatization using N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), and GC-MS analysis are all part of the procedure. Placket-Burman Design (PBD) and Central Composite Design (CCD) were utilized for the screening of FPSE's significant factors and their optimization, respectively, in the design of experiment-based optimization. For FAV, sol-gel polyethylene glycol (PEG) provided the highest extraction efficiency of all tested membranes. By GC-MS, the proposed method was found to be linear between 0.01 and 10 g mL-1 under ideal conditions. By GC-MS, the LODs and LOQs were as low as 0.001-0.0026 g mL1 and 0.003-0.0086 g mL1. Intra-day and between day precisions were under 5 and 10%, individually, showing great strategy accuracy.

Keywords: Fabric phase sportive extraction; Favipiravir; COVID-19

Introduction

Coronaviruses are single-stranded RNA viruses that are capable of infecting a wide variety of species, including humans, birds, and domestic and wild animals. Six distinct human coronaviruses have been identified since the 1960s: OC43, 229E, NL63, and HKU1 MERS-CoV SARS-CoV. The new form of coronavirus known as SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) is responsible for acute respiratory issues that are referred to as coronavirus disease (COVID-19). In December 2019, the COVID-19 pandemic was discovered in Wuhan, China, and quickly spread throughout the globe. In March 2020, the World Health Organization (WHO) declared it a pandemic. Nevertheless, infection rates continue to rise to previously unheard-of heights. As of April 1, 2020, there had been approximately 874,151 cases and 43,804 deaths as a result of the outbreak. As of June 10, 2022, the infection had tainted in excess of 530 million people and caused more than 6.3 million fatalities around the world [1-3]. Regardless of the endorsement of a few antiviral prescriptions, for example, favipiravir, umifenovir, remdesivir, and tocilizumab for various diseases, and progressing research on new choices in different districts, there are right now no particular antiviral meds supported for the treatment of SARS-CoV-2. Several nations, including Egypt, Italy, Saudi Arabia, the United Arab Emirates (UAE), Japan, Russia, India, and Turkey, have recently included favipiravir (FAV) as a potential therapeutic option in their management guidelines.

Through intracellular phosphoribosylation, which prevents the formation of viral proteins, it becomes active. It has also been investigated for the treatment of deadly diseases like Ebola, Lassa, and, most recently, SARS-CoV-2. FAV was found to increase viral clearance and improve chest CT scans in numerous clinical studies to determine its efficacy in the treatment of COVID-19 infections [4]. T-705-ribofuranosyl-5′-triphosphate (T-705-RTP) is the drug's active metabolite when it is ribosylated and phosphorylated by host cellular enzymes after being ingested. The enzyme RNA dependent RNA polymerase (RdRp) of influenza and many other RNA viruses is preferentially inhibited when T-705-RTP is incorporated into the virus RNA in low quantities. Due to the lack of reports in the literature, liquid chromatography is the method of choice for analytical chemists for the determination of FAV in various matrices and other applications. Five research papers focused on drug pharmacokinetics and pharmacodynamics studies established liquid chromatographic methods for determining FAV in plasma. The HPLC/UV method with isocratic elution was used in two additional methods. For the bioanalysis of FAV in a different way, LC-MS/MS was used. The cyclic voltammetry method for determining FAV in pharmaceuticals and urine has recently been reported.

Materials and Methods

Plasma was extracted from a blood sample by centrifuging it for ten minutes at 5000 rpm at the Rotary & Blood Bank Society Resource Centre in Chandigarh, India. Two female and one male healthy participant provided urine samples. The biological samples were kept at a temperature of less than 4 degrees Celsius until they could be used after slowly thawing. The Institutional Ethical Committee has issued approval number for the study. 1109/20. In India, two distinct FAV tablets were purchased from a local market and claimed to contain 800 and 400 mg of FAV per tablet, respectively [5-7]. To mimic drug-protein binding under physiological conditions, biological samples like urine, blood, and plasma were fortified with varying concentrations of FAV, ranging from 0.01 to 10 g mL1, homogenized by vortex agitation for 5 minutes, and incubated at 37°C for at least 30 minutes. These samples were utilized without any pretreatment, such as deproteinization or filtration, for the purpose of method validation. Unbleached cotton fabric made of 100 percent cellulose served as the substrate for the preparation of four distinct sol-gel sorbent-coated FPSE membranes. Sol-gel sorbents include sol-gel Carbowax 20M (sol-gel CW20M), sol-gel poly (tetrahydrofuran), sol-gel poly (ethylene glycol) 10,000, and sol-gel phenyl triethoxysilane (sol-gel PTES). Sol-gel solutions were prepared independently using an optimized formulation in order to

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prepare the cellulose sol-gel sorbent coatings. In a large centrifuge, 10 mL of sol-gel precursor methyltrimethoxysilane (MTMS), 10 mL of methylene chloride, 10 mL of acetone, 5 g of polymer/precursor, and 4 mL of aqueous trifluoroacetic acid (95 percent water) were added sequentially to create the sol solution.

Due to the presence of one amide group and one hydroxyl group in the FAV molecule, peak tailing and decreased sensitivity are both consequences. Underivatized FAV had a retention time of 8.27 minutes, according to the results. Derivatization of FAV is required to improve its chromatographic properties and reduce its polarity in order to overcome this limitation. Therefore, the most common silylation reagent, BSTFA with 1% TMCS, was used to derivatize FAV. Factors that influence the derivatization process, such as reaction time and temperature, must be controlled in order to achieve the highest possible yield; and the ratio of BSTFA to pyridine were made to work best [8]. The FAV-di-TMS derivative had a retention time of 9.96 minutes and no peak tailing. Additionally methyl pendant group that is connected to the silicon central atom of the molecule, MTMS provides the London dispersion type of weak intermolecular interaction. Sol-gel sorbents have meso- and micropores-filled sponge-like morphologies and are naturally porous. As a result, analyses are extracted because they are able to easily interact with the functional groups embedded in the sol-gel sorbent network. The sol-gel sorbent's porous structure also makes it easier for the solvent to quickly diffuse throughout its body, resulting in a more rapid and thorough elution of the extracted analysts [9]. Due to its unique planer geometry and the permeability of the sol-gel sorbent coated FPSE membrane, fabric phase sorptive extraction makes use of the extraction mechanisms of solid phase extraction (exhaustive extraction) and solid phase micro extraction (equilibrium-based extraction).

Conclusion

A novel FPSE–GC–MS method for the extraction of FAV from pharmaceutical formulations and biological matrices was developed and tested for the first time [10]. The innovative micro extraction device's flexible support, high sorbent loading, and large surface area made it possible for the analysis and sorbent to interact quickly. This resulted in excellent recoveries for the target analyst and a short extraction equilibrium time. FPSE eliminated the potentially error-prone step of protein precipitation from the sample preparation procedure. In FPSE, the removal of specific sample preparation procedures like solvent evaporation and sample reconstitution reduces the likelihood of analyse loss and error sources. Due to the strong chemical bond between the sorbent and the substrate, which gives the FPSE membrane great chemical and solvent stability, any organic solvent can be used as the eluant for analyse back-extraction. Using sol–gel short-chain PEG coated FPSE media for FAV extraction from pharmaceutical formulations and biological matrices proved to be a simple, highly efficient, and quick sample preparation method.

Declaration of competing interest

The authors declare that they have no competing financial interests

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