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An effective and economical method for the storage of plasma samples using a novel freeze-drying device

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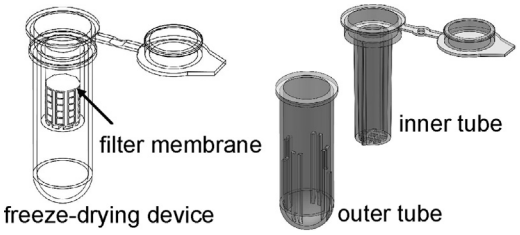
HIGHLIGHTS

- A novel freeze-drying device was built to store samples at the ambient temperature.
- An effective and economical method of sample storage was proposed using the device.
- The sample preparations integrated in the new storage method were simple and easy.
- This new method has been successfully applied to the PK studies of two drugs.

GRAPHICAL ABSTRACT



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ABSTRACT

Biological samples, especially plasma samples, are conventionally stored under freezing conditions to maintain sample integrity prior to the detections of analytes. However, the storage of samples in a low-temperature environment is electric energy consuming, and the preparation of samples prior to analytes detection may be complicated. In this work, an effective and economical method was proposed to freeze-dry the samples using a novel device to allow subsequent storage of samples at ambient temperature. The sample preparations integrated in the new method are simple and easy to follow. Analytes were directly extracted with the extraction agent before sample injections. This new method was validated with quality control (QC) samples of levetiracetam and mycophenolic acid (MPA), and it was also applied to the pharmacokinetic (PK) studies of both drugs in healthy volunteers. When QC samples were stored and prepared with the new method, the detections of analytes were accurate and repeatable, and the analytes maintained stability for a long time. The PK studies of levetiracetam and MPA in healthy volunteers showed that the PK parameters of analytes stored with the new method were consistent with those stored with the conventional method. In conclusion, this effective and economical method is a practical option in reality and can play a big role in clinical and scientific drug researches.

Abbreviations: PK, Pharmacokinetic; HPLC, High-performance liquid chromatography; LC-MS/MS, Liquid chromatography coupled with tandem mass spectrometer; DBS, Dried blood spots; DPS, Dried plasma spots; QC, Quality control; MPA, Mycophenolic acid; IS, Internal standards; GCP, Good Clinical Practice; RSD, Relative standard deviation.

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1. Introduction

In order to investigate the pharmacokinetic (PK) properties of drugs, many biological samples must be collected to measure the drug's concentrations in preclinical and clinical studies to profile the disposition of drugs in vivo [1,2]. An appropriate method to store those samples is essential for accurate concentration detection and reducing effects of drug decomposition [3,4]. Biological samples, especially plasma samples, are conventionally stored under freezing conditions prior to analysis of analyte concentrations [5–7]. Generally, samples may be kept at −20 °C for short-term storage, and at −80 °C for long-term storage. Some samples must be stored at −80 °C for several years in case of an audit from sponsors or regulatory agencies. The traditional storage method is convenient and straightforward, typically involving the storage of samples in a refrigerator or freezer after a simple preparation, such as a centrifugation step. However, its dependence on storage in a low-temperature environment is electric energy consuming and may include inconvenient transport of samples. Moreover, prior to detection of the analytes with appropriate analytical methods such as high-performance liquid chromatography (HPLC) or liquid chromatography coupled with tandem mass spectrometer (LC-MS/MS), additional processing is required for some preparations such as liquid–liquid extraction, protein precipitation, and others [8–11]. These preparation steps can be complicated and uneconomical. Liquid–liquid extraction involves a series of operations including analyte extraction with organic regents, evaporation, and reconstitution with the mobile phase, thus this process may consume intensive amounts of solvent and increase the risk of mistakes during processing due to the high number of steps [9]. Similarly, preparing samples by the protein precipitation sometimes needs to take other actions to reduce the influence of matrix effect on the detections [11]. In such cases, use of a conventional storage method may complicate the detection of analytes and increase the cost.

Recently, two new methods including dried blood spots (DBS) and dried plasma spots (DPS) have been proposed for the storage of blood and plasma samples [12–15]. Samples stored by these methods can be maintained at ambient temperature and be transported conveniently. These technologies have been recently applied for the screening of neonatal diseases and metabolism related diseases [16,17]. Nonetheless, both technologies are imperfect and accuracy can be influenced by the conditions of spotting, including temperature, humidity, volume of blood or plasma, and other factors. In addition, the volume of samples in DBS and DPS is so small that highly sensitive detection methods, such as LC–MS/MS, are needed [14,15,17]. All these imperfections limited the extension of DBS and DPS to more widespread practice.

In order to reduce costs and simplify the process of samples storage, a novel freeze-drying device as well as a new sample storage method has been developed. The applicability of this device and method has been successfully validated by applying it to the storage of quality control (QC) samples of levetiracetam and mycophenolic acid (MPA), which were conventionally stored in a low-temperature freezer and prepared by liquid–liquid extraction or protein precipitation. Finally, the proposed method was applied to the PK studies of levetiracetam and MPA in healthy volunteers.

2. Methods

2.1. Materials and machines

The detection of analytes by HPLC was performed on the Agilent 1200 HPLC system comprising a G1322A degasser, a G1311A quat-pump, a G1329A autosampler, a G1316A column oven and a G1314B UV-detector (Agilent, USA). An Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm, Agilent, USA) equipped with a Security-Guard C18 column (4.0 mm × 3.0 mm, 5 μm, Phenomenex, USA) was used to separate analytes.

The levetiracetam standard (99.7%, Chongqin fuan pharmaceutical Co., Ltd, China) and the MPA standard (99.7%, Wuxi fuqi pharmaceutical Co., Ltd, China) were provided by the manufacturers. Secnidazole (98.0%, Beijing chemical reagent company, China) and phenacetin (98.0%, Sigma, USA) were used as the internal standards (IS) for levetiracetam and MPA, respectively. Methanol and acetonitrile were of HPLC grade (Tedia, USA), and phosphoric acid (Sinopharm Chemical Reagent, China) was guaranteed reagent. Other chemicals were of analytical grade. Deionized water was purified using the Purelab classic system (ELGA Labwater, China).

2.2. Novel method for sample storage and preparations

2.2.1. Freeze-dying device

The novel freeze-drying device consisted of two tubes. The inner tube was used for intercepting the analytes using a membrane, and the outer tube was for reserving the filtered samples (Fig. 1). The inner diameters of the inner tube and outer tube were 7 mm and 10.2 mm, respectively, and the heights of the tubes were 31.6 mm and 35 mm, respectively. The membrane placed at the bottom of the inner tube was made of silicone rubber and fiberglass. It had a narrow distribution of pore size and the hydrophily of the materials was moderate. The plasma could evenly diffuse over the surface of the membrane and then the analytes were intercepted by being absorbed over the membrane when the plasma sample was added into the inner tube and ultrasonically blended for 5 min. The filtered sample then flowed out the inner tube through the bottom pore with a shape of "#" and was reserved in the outer tube. Finally, the inner tube was removed from the outer tube and placed into the vacuum freeze dryer to be freeze-dried. The volume of the plasma sample stored in each tube was 200 μL.

2.2.2. Freeze-drying process

The freeze-drying program was designed according to the settings listed in Table 1. The tubes were first incubated at −40 °C in the freeze drier for 3 h to pre-freeze the samples. Then the samples were heated to −37 °C and the vacuum pump began operation. The temperature in the freeze drier was programmed to increase slowly and reached 20 °C eventually. After all programmed settings were completed, the freeze-dried tubes were removed to low-gas-permeable and zip-lock plastic bags to be stored at ambient temperature until being assayed.

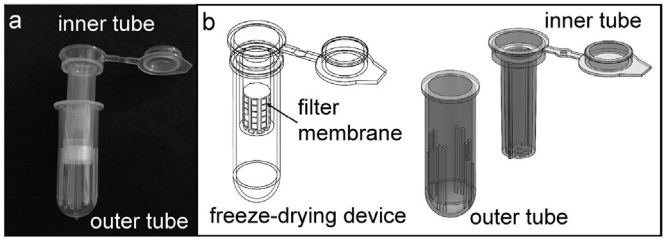

Fig. 1. The photo of the novel freeze-drying device (a) and the illustration of the structure of the device (b). The proposed device consisted of an inner tube and an outer tube. Samples were added into the inner tuber and the volume of the sample was 200 μL. The membrane placed at the bottom of the inner tube was used for filtering the plasma sample to intercept the interest analytes. The filtered sample then flowed out the inner tube through the bottom pore with a shape of "#" and was reserved in the outer tube. The inner tube was finally removed from the outer tube and placed into the vacuum freeze dryer to be freeze-dried.