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Mass spectral study of storage conditions and paper substrates on the degradation and analytical sensitivity of therapeutic drugs in dried blood spots

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ABSTRACT

Dried blood spot sampling technique has been a prevalent approach in therapeutic drug monitoring due to its numerous advantages over conventional blood or plasma sampling. But the effects of storage conditions, drying time in air and paper substrates on the stability and analysis sensitivity of therapeutic drugs are still lack. Herein we systematically investigated the effects of storage conditions and paper substrates on the appearance of dried blood spots and stability of different therapeutic drugs by using a facile paper spray mass spectrometry. The results demonstrated using silica coated paper SG81 favored to get an abundant signal due to the unique elution behavior of target compounds in dried blood spots relative to that with grade ET31 and grade 1 paper substrates. By examining the effects of light illumination and air on the stability of therapeutic drug, it reveals that both have a similar impact on the degradation of the tested drugs, whereas storing the samples in a zip-foiled bag could effectively suppress the degradation of the analytes in dried blood. The analysis sensitivity of different drugs in blood samples was also compared under different storage conditions. An improvement of 2–10-fold analysis sensitivity was obtained for these drugs when the DBS were stored in a zip-foiled bag relative to that exposure to air.

1. Introduction

Dried blood spots (DBS) were utilized for the first time on human blood in early 1960s by Guthrie and Susi for detection of phenylketonuria in large populations of newborn infants [1]. Since then, DBS sampling technique has been a prevalent way to collect and store blood samples due to its numerous advantages (*e.g.*, less invasiveness, small blood volume, simple storage method, cheap sample shipment, less risk of blood borne pathogens) over conventional blood or plasma sampling [2,3]. At present this technique is not only used in newborn screening for metabolic disorders, but also has been extended to many fields including epidemiological [4], toxicokinetics [5], pharmacokinetic studies [6,7], diagnostic screening [8–10] and therapeutic drug monitoring [11–14]. Among them, great challenges have been posed to the analysis of therapeutic drugs in DBS although a number of studies have been well documented. For example, the instability of drugs in DBS samples during transportation and storage possesses challenge in the interpretation of results of analysis due to the short half-life and the effects of light, temperature, pH, water, or others. Another challenge is that this blood sampling has to be used in conjunction with analytical techniques capable of detecting the low amounts of analytes present in few microliters of blood.

Over the past decades, considerable effort has been devoted to the investigation of various effects on the stability of different drugs in DBS. For example, Prieto et al. [15] investigated the effect of storage temperature on the stability of nitisinone in DBS, and observed that this drug was stable in plasma at 4 °C for a period of one and a half months, and there was a slow degradation at room temperature and it reached a degradation of around 3% after only two days. Sadilkova et al. [16] found that tacrolimus, sirolimus, and cyclosporin A in DBS quality control samples were stable for at least 30 days at −20 °C, 4 °C, and 25 °C, and stability of patient DBS samples was at least 5 days at temperatures up to 60 °C, except for sirolimus where degradation was observed at 60 °C within 24 h. In the drug degradation process, it involves hydrolysis, oxidation, thermolysis or photolysis of the drug substances. By evaluating the commercially available DBS cards, Liu et al. [17] found that an unstable drug candidate KAI-9803 degraded during the drying process because of thiol-disulfide exchange at physiological pH. Based on this reason, a facile strategy was suggested to suppress this drug degradation by rapidly lowering the pH of the

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spotted blood sample. In order to prevent the influence of moisture on the analytes of interest in DBS, Mei et al. [18] regarded that it was very important to dry blood spot specimens completely before storage or transport, because moisture may harm the specimens by inducing bacterial growth or altering the elution time of the specimen. Blood spot specimens should be dried for at least 3 h over an open nonabsorbent surface at 15–22 °C. To minimize the effects of light illumination, air oxidation and humidity, Faller et al. [19] stored DBS in zip-foiled bags with a desiccant pack. Although great advance has been made in improving the stability of therapeutic drugs in DBS, it was found worthwhile to provide an overview of the storage conditions (*e.g.*, time, temperature, and paper substrate) on the stability of drugs in DBS. More importantly, liquid chromatography–mass spectrometry is currently the most widespread used technique for DBS analysis due to its unique performance in quantitation analysis. Such an analytical procedure, however, is time-consuming and inconvenient, and especially requires around one hundred microliter aliquots of whole blood for spotting, unfavorable to a small-volume blood sample. To overcome these issues, herein we employ a facile analytical technique paper spray mass spectrometry [14,20] by using only 2 μL blood sample per DBS to systematically investigate the effects of various storage conditions and paper substrates on the stability and analytical sensitivity of therapeutic drugs in DBS. In this study, the blood samples were spiked with different therapeutic drugs, including amitriptyline, clozapine, amisulpride, quetiapine, risperidone, aripiprazole and verapamil, prior to spotting on Whatman grade 1, grade ET31 and grade SG81 paper substrates. Detailed study was carried out on the impacts of storage conditions such as light, air, temperature and storage time as well as drying time in air on the drugs of interest in DBS.

2. Experimental

2.1. Preparation of whole blood samples

The standards (Sigma–Aldrich, St. Louis, MO) used for these experiments were prepared as follows: therapeutic drug solutions were prepared by dilution of stock solutions into 1:1 methanol/water for amitriptyline, clozapine, amisulpride, quetiapine, risperidone, aripiprazole and verapamil with a concentration of 1 mg mL^{-1}. The standard solutions were then spiked into whole blood samples by pipetting 5 μL of the standard into 495 μL of whole blood with a drug concentration of 10 μg mL^{-1}. The samples of lower concentrations of therapeutic drugs were prepared with a series of dilutions, each with a small volume of therapeutic drug sample at higher concentration and large volume of whole blood. For example, the blood sample with 1000 ng mL^{-1} amitriptyline was prepared with 40 μL of the prepared 10 μg mL^{-1} blood sample and 360 μL of whole blood. The concentrations of the therapeutic drugs in the final blood samples were 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, 50, 100 and 1000 ng mL^{-1} or as otherwise indicated in the figures.

2.2. Preparation of DBS samples

DBSs were prepared by spotting a fixed volume (2 μL) of whole blood sample onto Whatman grade 1, grade ET31 and silica coated paper substrate grade SG81 (GE Healthcare Bio-Sciences Corp., Westborough, MA, USA) and drying for different periods at room temperature or other conditions as indicated. The samples were stored at room temperature in a sealed bag or otherwise stated. It should be pointed here that no desiccant was used for storing the DBS samples because little effect on the humidity in the sample storage bags was observed after comparison between with and without desiccant (data not shown).

2.3. Paper spray analysis

The procedure for paper spray experiment has been described in previous reports [14,20]. Briefly, the paper substrate spotted with blood sample was cut into a triangle with a height of 10 mm and a base width of 5 mm. A copper clip was used to hold the paper and to apply a high voltage (3.5 kV). The distance between the paper triangle tip and the mass spectrometer inlet was about 5 mm. Spray solvent 9:1 methanol/water (25 μL) was then added to the base of the paper triangle followed by application of a high voltage. The selection of solvent for paper spray was based on the previous studies [14,21]. All experiments on paper spray were carried out with a TSQ Quantum Access Max mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA). Mass spectra were recorded in the positive ion mode with a capillary temperature of 270 °C. The identification of analyte ions was confirmed by tandem mass spectrometry (MS/MS) using collision-induced dissociation (CID). The signal intensity was averaged over the entire period of the selected reaction monitoring (SRM) with the paper spray. The SRM and instrumental parameters used for the drug compounds were as follows: amitriptyline, m/z 278 → 233; tube lens, 66 V; collision energy, 17 V; clozapine, m/z 327 → 270; tube lens, 75 V; collision energy, 22 V; D8-clozapine, m/z 335 → 275; tube lens, 76 V; collision energy, 24 V; amisulpride, m/z 370 → 242; tube lens, 74 V; collision energy, 26 V; quetiapine, m/z 384 → 253; tube lens, 77 V; collision energy, 21 V; risperidone, m/z 411 → 191; tube lens, 77 V; collision energy, 26 V; aripiprazole, m/z 448 → 285; tube lens, 89 V; collision energy, 25 V; verapamil, m/z 455 → 165; tube lens, 91 V; collision energy, 26 V. The used bovine whole blood was purchased from Lanzhou Institute of Biological Products Co., Ltd. (Lanzhou, China).

3. Results and discussion

In order to study the stability of therapeutic drugs in DBS, three different types of papers including Whatman grade SG81, grade ET31 and grade 1 were used as substrates for spotting blood samples spiked with drugs of interest. Silica coated paper namely grade SG81 was selected as a paper substrate for DBS analysis owing to its higher drug elution efficiency than grade 4 and ET31 papers [14]. To make a comparison with grade SG81 (0.27 mm thick), a similar thickness paper grade 1 (0.18 mm thick) was used for DBS. Grade ET31 was utilized herein because it is a paper substrate for preparing blood card in Whatman (personal communication). After spotting the prepared whole blood sample on the above papers and storing them under normal laboratory conditions, it was noted that the appearance of the DBS was changing from day to day. As shown in Fig. 1a, the color of DBS for the three papers changed from fresh red to pink, and then to black red with varying the storage time from just applying the blood on paper to 36 h, which is similar to the report by Denniff and Spooner [22]. Among the three papers, SG81 paper demonstrates a more obvious change than grade ET31 and grade 1. Also the color gradually became deeper and deeper from SG81 to grade 1 followed by ET31. The color variation between them could be attributable to the different surface properties and paper thickness as well as the loss of moisture during storage [22].

With the appearance variation of the DBS, it was also found that the contents of therapeutic drugs gradually decreased with extension of storage time. To gain an insight into the effects of storage time and paper substrate on the levels of drugs in DBS, verapamil was used as a probe molecule. As shown in Fig. 1b, the signal intensity of verapamil decreased step by step with the storage time from 0 to 72 h for all the three types of paper substrates. For example, the degradation percentage for both SG81 and ET31 was around 27%, and the value for grade 1 was as high as 35%. But careful