



Development of a hematocrit-insensitive device to collect accurate volumes of dried blood spots without specialized skills for measuring clozapine and its metabolites as model analytes



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ABSTRACT

Dried blood spots have been used as alternatives to traditional plasma and serum samples. We have now developed new devices, named volumetric absorptive paper disc (VAPD) and mini-disc (VAPDmini), to collect accurate volumes of dried blood spots in a simple manner and without the need for additional instruments. VAPD consists of a filter paper disc and a filter paper sheet with holes slightly larger than the disc. The disc is fixed in one such hole without direct contact with the filter sheet. VAPDmini is a scaled-down version of the same device. When several drops of whole blood are applied, the disc becomes saturated and any excess sample is absorbed by the surrounding filter sheet. Accuracy and precision of sampling were assessed by determining the levels of clozapine and its metabolites as target analytes by liquid-liquid extraction and high-performance liquid chromatography with coulometric detection. In addition, differences in analyte recovery were within $\pm 15\%$ for all analytes in samples with 30–60% hematocrit, suggesting that VAPD and VAPDmini are insensitive to hematocrit for the analytes tested. The devices were also validated for analyte concentrations in the range 50–1000 ng/mL, and the limit of detection and lower limit of quantification were 5–17 ng/mL and 15–51 ng/mL, respectively. Intra- and inter-day precision ranged from 3% to 13%, whereas accuracy ranged from a -14% to 12% bias. Analytes were stable in the devices for at least 2 weeks at room temperature. Collectively, these results indicate that sampling using VAPD and VAPDmini is comparable to conventional hole punch sampling of entire dried blood spots, even for samples obtained from patients treated with clozapine. Importantly, the devices were also found to be suitable for sample self-collection.

1. Introduction

Dried blood spots collected on filter paper have been widely used for bioanalysis. Collection of dried blood spots has numerous advantages over traditional methods, including minimally invasive sampling, small sample volumes, and convenient storage and transport [1]. The dried blood spot method has been used for self-sample collection. However, specialized devices are required for quantitative self-sampling, because the volume percentage of red blood cells in the sample (hematocrit) significantly affects spot size and homogeneity [1–4], and thus, the accuracy and precision of the subsequent assays.

To overcome hematocrit effects, several procedures to collect accurate blood volumes on filter paper discs or tips have been developed, although entire blood spots may have to be analyzed to minimize the

effects of hematocrit [1]. For example, a technique in which a fixed volume of whole blood is applied to pre-cut discs punched from commercially available cards has shown good accuracy and precision [5]. However, this approach requires pipettes. To eliminate the need for additional devices, such as pipettes or microcapillaries, and to effectively eliminate the effects of hematocrit, a volumetric absorptive microsampling technique has been proposed [6–8], in which an absorbent polymeric tip is dipped into a drop of blood or into pooled blood. We have now combined the principles of both approaches to develop new devices, named volumetric absorptive paper disc (VAPD) and mini-disc (VAPDmini), which enable collection of accurate blood volumes. VAPD consists of a filter paper disc and a filter paper sheet with holes slightly larger than the disc. The disc is fixed using adhesive tape in one such hole without direct contact with the filter paper sheet. VAPDmini is a

Abbreviations: VAPD, volumetric absorptive paper disc; VAPDmini, volumetric absorptive paper mini-disc; HPLC-CD, high-performance liquid chromatography with coulometric detection; %RE, percentage relative error; %RSD, percentage coefficient of variation; LOD, limit of detection; LLOQ, lower limit of quantification

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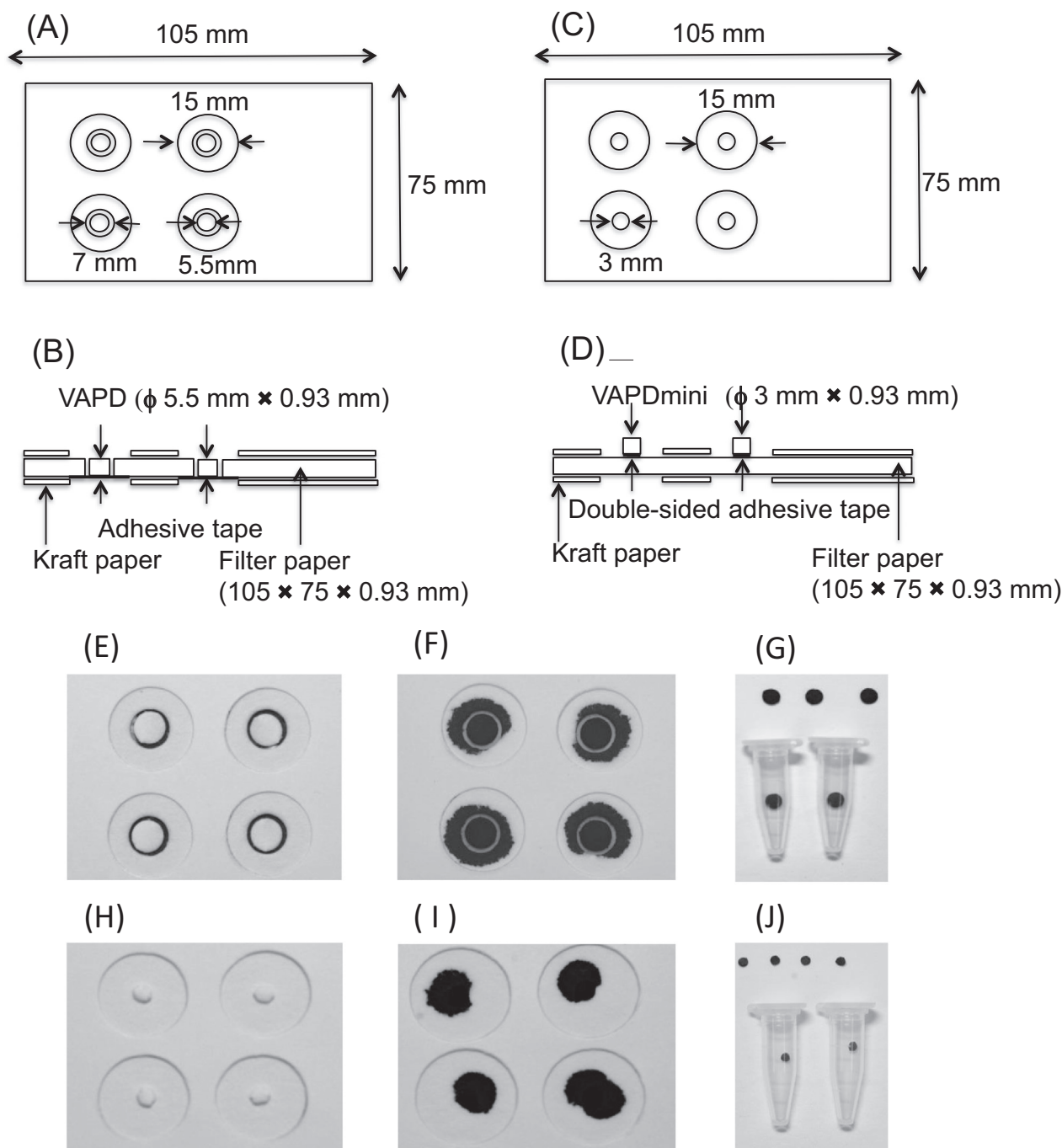


Fig. 1. Upper (A, C) and lateral (B, D) views of volumetric absorptive paper disc (VAPD) (A, B) and VAPDmini (C, D). VAPD (E) and VAPDmini (H) were loaded with blood (F, I) and removed (G, J) after drying.

scaled-down version of the same device. When several drops of whole blood are applied, the disc becomes saturated, and any excess sample drains into and is absorbed by the surrounding filter sheet. In initial experiments, we found that blood flows rapidly through the discs, so that samples with low hematocrit were not retained. Thus, a thicker filter paper was selected to slow sample flow, and thereby to capture sufficient volumes regardless of hematocrit levels.

VAPD and VAPDmini were validated using clozapine and its metabolites as model analytes. Clozapine, the prototype atypical antipsychotic, is known to have superior efficacy against treatment-resistant schizophrenia [9]. However, monitoring of serum levels of clozapine and its active metabolite N-desmethylclozapine is strongly recommended by the AGNP Workshop for Neuropsychopharmacology

and Pharmacopsychiatry owing to their narrow therapeutic range and serious toxic side effects [10,11]. Of note, clozapine and its metabolites have been recently quantified in dried blood spots by microextraction and high-performance liquid chromatography with coulometric detection (HPLC-CD) [12], and by solvent extraction and ultra HPLC-tandem mass spectrometry [13]. Furthermore, the stability of the clozapine metabolite clozapine N-oxide has also been assessed in punched dried blood spots by solvent extraction and HPLC-tandem mass spectrometry [14]. In those studies, dried blood spots proved to be reliable alternatives to conventional serum and plasma specimens, although the methods themselves were not validated to account for the possible effects of hematocrit. Thus, we aimed not only to develop, but also to validate a simple and accurate HPLC-CD method to quantify clozapine

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