Introduction: Generally blood samples for homocysteine detection are recommended to be centrifuged immediately to separate plasma in order to avoid continuous synthesis in blood cells. The use of a micro plasma collection card may improve sampling by automatic separation of plasma instantly. The goal of this study was to compare a micro plasma collection method with routine sampling to explore applications of Dried Plasma Spot (DPS) for homocysteine determination by using liquid chromatography tandem mass spectrometry (LC-MS/MS) method.

Methods: After a standard micro plasma collection procedure, DPS was extracted and precipitated to analyze homocysteine by a previously validated LC-MS/MS method. Plasma volume factor was acquired by calculating the ratio of homocysteine concentration between DPS and wet plasma from a same individual (n=165). Hematocrit and homocysteine concentration were studied to evaluate the impact on plasma volume factor. Imprecision, recovery and stability of DPS were also explored.

Results: The assay was linear from 0.5-45 μmol·L⁻¹ with good precisions and accuracies. The extraction recovery and matrix effects for dried plasma spots were more than 97% and 98% after internal standard normalization, respectively. It was reproducible for retaining homocysteine in dried plasma spots and kept stable for 30 days. The plasma conversion factor was 7.77±0.7% by calculating the ratio of homocysteine concentration between dried plasma spots and wet plasma (n=165). Neither hematocrit nor homocysteine concentration affected the plasma conversion factor as long as the hematocrit was within the normal range.

Conclusions & Discussion: The results support the clinical usefulness of the dried plasma spots as a convenient and stable biological matrix for testing homocysteine.

Abstract

Introduction

Homocysteine is generally accepted as an independent risk factor for atherosclerosis and cardiovascular disease. Accurate and reliable quantification of homocysteine in plasma samples is essential in clinical practice to detect hyperhomocysteinemia. Storage of plasma may lead to increases in oxidized forms of homocysteine and decreases in tHcy levels. Similarly, a time- and temperature-dependent release of tHcy from erythrocytes leads to an artificial increase of tHcy in the plasma. Thus, it is necessary to separate erythrocytes from blood as soon as possible after blood drawing.

The dried blood spot is used to detect homocysteine for it’s less invasive sampling and enhanced stability while the poor quantitation limits its use. Here we present an application of micro plasma collection card for homocysteine determination by preparing a dried plasma spot (DPS). The goal of our study is to evaluate the clinical applicability of DPS in homocysteine determination by an established LC-MS/MS method.

Method

a. Preparation of DPS: A drop of blood (~ 20 μL) was dropped onto the test area of the micro plasma collection card until the control spot turned red. After three minutes, the top layer of the card was peeled off. The lower layer was dried under ambient temperature for 15 minutes and then preserved or delivered to the lab at a room temperature.

b. Extraction and Analysis: DPS in the lower layer (usually collect a constant plasma volume of 2.52±0.03 μL) was taken out with mix 25 μL extractant (a substitute of matrix containing very low level of endogenous homocysteine ~0.1 μmol·L⁻¹) including internal standard (5 μmol·L⁻¹, homocysteine-d₄). After extraction, supernatant was separated by a UPLC elution procedure before quantification by mass spectrometry.

c. The validation and investigation of DPS: Before further studies were carried out, the LC-MS/MS method for determining homocysteine in DPS was validated to assure its accuracy, precision as well as its recovery and matrix. Besides, the reproducibility and stability of homocysteine in DPS were also verified.

d. Plasma Conversion factor: Plasma Conversion Factor was used to convert the concentration of the drug on DPS to plasma. It was acquired by calculating the ratio of homocysteine concentrations in DPS and in wet plasma (See test program below). Here, the influence of hematocrit and concentration of homocysteine to plasma conversion factor was both considered.

Figure 2. Test program of Plasma Conversion Factor

1. Whole blood was drawn from venipuncture
2. One drop of blood (~ 20 μL) was prepared DPS
1. Another 50 μL blood was used for detecting hematocrit
2. The remainder was centrifuged to separate plasma again for quantifying homocysteine Concentrations

Figure 3. Homocysteine stability in DPS and wet plasma at ambient temperature (n=6)

A. Reproducibility and stability of DPS: The reproducibility of DPS was determined by assaying 6 replicates from the same individual (%CV did not exceed 3.7%). When blood was placed at ambient temperature, the plasma concentration of homocysteine increased rapidly during 48 h. While it kept stable in DPS samples for 30 days (see Figure 3).

B. Plasma conversion factor and influence of haematocrit: The plasma conversion factor, calculated from the ratio of homocysteine concentration, was 7.77±0.7% (n = 75). It was shown that the plasma conversion factor of homocysteine could be stable when HCT ranged from 32.5% to 54% (Figure 4).

C. Homocysteine concentration in human blood: the homocysteine concentrations calculated from the DPS showed an excellent correlation with those measured in wet plasma when homocysteine concentrations were less than 156 μmol·L⁻¹.

Figure 4. Influence of haematocrit on plasma conversion factor of homocysteine (n=75)

Results

References


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