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Identification of potential sphingomyelin markers for the estimation of hematocrit in dried blood spots via a lipidomic strategy

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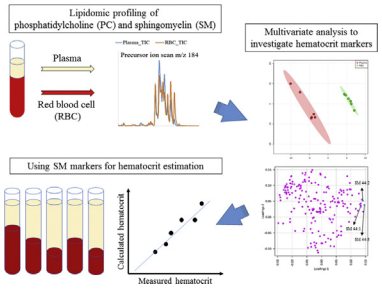
HIGHLIGHTS

- We developed a convenience strategy for HCT estimation via LC-ESI-MS platform.
- Lipidomics is a promising strategy for screening HCT estimation markers.
- PCs and SMs profiling of membrane extract was performed in this study.
- We successfully identified three HCT estimation markers.

GRAPHICAL ABSTRACT



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ABSTRACT

The dried blood spot (DBS) strategy is a convenient and minimally invasive approach to blood sampling. Due to its various advantages, this sampling technique has drawn significant attention in recent years. Hematocrit (HCT)-associated bias is one of the main obstacles that hinder wider DBS application in clinical practice. An accurate HCT estimation method could help calibrate HCT-associated bias and improve the quantification accuracy. This study used a lipidomics profiling strategy to identify HCT estimation markers using liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS), which provided advantages including the potential for the simultaneous measurements of target drug and HCT values. Three sphingomyelins (SMs), specifically SM 44:1, SM 44:2, and SM 44:3, were identified as potential HCT estimation markers. The proposed estimation markers were applied to 54 DBS samples collected from two sets of patients. The analytical results revealed that the estimation errors for all of the HCT values were less than 20%, which demonstrated the feasibility of using the proposed markers to estimate the HCT values for the DBS samples. We suggest that the proposed HCT markers could provide a new strategy for HCT estimation with higher convenience using an LC-ESI-MS platform, which could contribute to wider DBS applications in clinical practice. We also demonstrated that lipidomics is a promising strategy for the discovery of HCT estimation markers in DBS samples.

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1. Introduction

The idea of using a cellulose paper card for blood collection has a long history that can be traced back to 1913 [1,2]. The dried blood spot (DBS) sampling strategy has been widely applied in clinical applications because it provides a minimally invasive approach for acquiring a relatively small volume of blood sample for newborn disease screening [3–6]. The advantages of the DBS sampling technique include the requirement for only a small amount of blood, easier transportation from the sampling site to analytical laboratory, and a lower risk of exposure to biohazards during shipment [7–9]. Additionally, the spots remain stable under suitable storage conditions for months to years after drying [10,11]. The sample preparation procedure at the sampling site is also much simpler than conventional venous blood sampling approaches [7–9]. However, the clinical utility of this sampling technique has been relatively low since its introduction. One of the main obstacles for the wider application of DBS sampling techniques is the small amount of sample on the card, which makes the detection of target compounds challenging. As high sensitivity mass spectrometry has become more affordable, wider application of this simple and cost-effective technique has become feasible. The DBS sampling technique has been successfully applied to therapeutic drug monitoring and clinical trials for various drugs.

Even though the sensitivity problem has been overcome by improvements in mass spectrometry instrumentation, the effect of blood volume- and hematocrit (HCT)-associated variations are still two of the main issues that restrict wider application of the DBS strategy. To overcome the blood volume problem, several strategies, such as taking a fixed-diameter subsample and using Mitra or volumetric absorptive microsampling methods, have been proposed. Although blood volume variations can be overcome by taking a fixed-diameter subsample from DBS samples, the quantification accuracy of this strategy may still be affected by different spreading rates caused by different HCT values [12]. The whole-spot extraction method is less affected by HCT-associated spreading errors, and the postcolumn infusion-internal standard method has been used to correct for the blood volume variations in the whole-spot extraction method [13–15]. Other forms of HCT-associated bias include extraction recovery differences and different matrix effects for each blood spot [16,17]. For clinical drug analysis, the HCT value is also used for adjustments before correlating drug concentrations in plasma and blood [3,18,19]. Therefore, it is important to estimate and correct for HCT-associated bias.

Various methods have been developed to reduce HCT-associated bias or to estimate the HCT values [20]. Abu-Rabie et al. have proposed several strategies for the addition of an internal standard (IS) to DBS samples (e.g., pre-loading the paper with the IS or spraying the paper with the IS prior to DBS desorption) [21,22]. Their methods have been demonstrated to effectively resolve the HCT-based recovery bias and quantification errors caused by matrix effects. However, the stability problem of pre-loading the paper with IS needs to be considered before it can be used for actual clinical applications [23]. To obtain HCT values for the correlation of drug concentrations in plasma and blood, near-infrared spectroscopy methods, noncontact diffuse reflectance spectroscopy methods, and potassium-based algorithms have been proposed [12,17,24,25]. These approaches could not only calibrate the HCT-associated blood volume variations but also correct for variations caused by the analyte distribution between the blood and plasma. According to these studies, components with consistent levels in red blood cells (RBCs) have great potential as markers to improve the accuracy of HCT estimation. Capiau et al. had proposed using potassium as an HCT estimation marker [17]. However, the potassium concentration should be analyzed with a potentiometer, which cannot be performed simultaneously with drug concentration measurements that generally use a mass spectrometer. Therefore, this method may have the problem of DBS sample consumption and require additional sample preparation steps. The same group has reported that estimation of the hemoglobin present in RBCs using noncontact diffuse reflectance spectroscopy is potentially useful as a method for HCT correction [12]. However, the unstable properties of hemoglobin may result in uncertainty in the HCT estimation, and the choice of the best wavelength to ensure stability does not contribute to the uncertainty becomes an important issue. Considering the limitations of current methods, this study aimed to develop a new strategy for HCT estimation using liquid chromatography-mass spectrometry (LC-MS) analysis.

Lipidomics focuses on the study of the cellular lipidomes and the organizational hierarchy of lipid and protein constituents that mediate life processes [26]. The mammalian cell membrane is mainly constructed of phospholipids, and the phospholipids in red blood cells should be promising for identifying potential markers for HCT estimation. Because liquid chromatography-mass spectrometry is the most widely used platform for lipidomics research, it is very possible that the HCT markers identified using a lipidomics strategy could be measured at the same time as the target drug, which would make the markers very convenient in the clinical laboratory. It has been proposed that similar membrane lipid composition is needed to maintain cell structure [27,28]. We therefore hypothesized that certain lipid species should remain relatively constant between individuals and that their levels should show large differences between RBCs and plasma. Accordingly, this study applied a lipidomics profiling strategy to investigate the most abundant membrane lipids including phosphatidylcholine (PC) and sphingomyelin (SM) components of the RBC membrane as potential HCT estimation markers.

In this study, we proposed three SMs as potential HCT estimation markers using a liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) lipidomics profiling strategy. In contrast to other published HCT estimation methods, this method provides the advantages of being able to simultaneously analyze the HCT and target drug on the same platform without needing additional analytical instruments. The proposed SM markers were applied to estimate the HCT values in 54 clinical samples. By comparing the HCT values obtained using the typical venous blood HCT measurement method that utilizes the Sysmex XE-5000 automated hematology system, the proposed SMs were demonstrated as promising markers for HCT estimation in DBS samples.

2. Experimental section

2.1. Chemicals and materials

Supragradient HPLC-grade methanol was purchased from Scharlau Chemie (Sentmenat, Barcelona, Spain). Formic acid (99%) and ammonium acetate were purchased from Sigma-Aldrich Co. (St. Louis, MO). Methanol (MeOH) and isopropyl alcohol were obtained from J.T. Baker (Phillipsburg, NJ). The 1-(10Z-heptadecenoyl)-sn-glycero-3-phosphocholine (LPC17:1), N-stearoyl-D-erythro-sphingosylphosphorylcholine (SM (d18:1/18:0)), and N-oleoyl-D-erythro-sphingosylphosphorylcholine (SM (d18:1/18:1)) were purchased from Avanti Polar Lipids (Alabaster, AL). Whatman 903 Protein Saver cards (Maidstone, UK) were used for the collection of blood samples (DBS samples). The punch was purchased from a local store and had a size of 3 mm.