Talanta 150 (2016) 681–689

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Influence of the collection tube on metabolomic changes in serum and plasma

M.A. López-Bascón [a,b,c], F. Priego-Capote [a,b,c,*], A. Peralbo-Molina [a,b,c], M. Calderón-Santiago [a,b,c], M.D. Luque de Castro [a,b,c]

[a] Department of Analytical Chemistry, University of Córdoba, Annex C-3, Campus of Rabanales, Córdoba, Spain
[b] University of Córdoba Agroalimentary Excellence Campus, ceiA3, Córdoba, Spain
[c] Maimónides Institute of Biomedical Research Maimónides (IMIBIC), Reina Sofía Hospital, University of Córdoba, Córdoba, Spain

ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form
25 December 2015
Accepted 30 December 2015
Available online 31 December 2015

Keywords:
GC–TOF/MS
Metabolomics
Plasma
Polymeric gel tubes
Sampling tubes
Serum

ABSTRACT

Major threats in metabolomics clinical research are biases in sampling and preparation of biological samples. Bias in sample collection is a frequently forgotten aspect responsible for uncontrolled errors in metabolomics analysis. There is a great diversity of blood collection tubes for sampling serum or plasma, which are widely used in metabolomics analysis. Most of the existing studies dealing with the influence of blood collection on metabolomics analysis have been restricted to comparison between plasma and serum. However, polymeric gel tubes, which are frequently proposed to accelerate the separation of serum and plasma, have not been studied. In the present research, samples of serum or plasma collected in polymeric gel tubes were compared with those taken in conventional tubes from a metabolomics perspective using an untargeted GC–TOF/MS approach. The main differences between serum and plasma collected in conventional tubes affected to critical pathways such as the citric acid cycle, metabolism of amino acids, fructose and mannose metabolism and that of glycerolipids, and pentose and glucuronate interconversion. On the other hand, the polymeric gel only promoted differences at the metabolite level in serum since no critical differences were observed between plasma collected with EDTA tubes and polymeric gel tubes. Thus, the main changes were attributable to serum collected in gel and affected to the metabolism of amino acids such as alanine, proline and threonine, the glycerolipids metabolism, and two primary metabolites such as aconitic acid and lactic acid. Therefore, these metabolite changes should be taken into account in planning an experimental protocol for metabolomics analysis.

1. Introduction

Human blood is the most common biofluid used by clinicians [1]. The main reasons justifying the clinical applicability of blood are its minimally invasive sampling [2], its homogeneity as compared to saliva or urine, which are strongly influenced by the collection volume [3], and its direct relationship with systemic changes in the metabolome [4,5]. Nevertheless, blood presents several limitations ascribed to its complicated and variable composition, as it contains approximately 4000 metabolites covering a wide range of concentrations and chemical diversity [6].

Two types of samples are obtained from blood, plasma and serum, which are used to evaluate various biochemical parameters demanded in the clinical field [4,5,7,8]. The collection of serum or plasma depends on allowing or blocking coagulation, respectively. During coagulation, fibrin clots are formed, then separated by centrifugation from serum together with blood cells and related coagulation factors, while platelets release proteins and metabolites into serum. On the other hand, plasma is obtained by addition of an anticoagulant (EDTA, heparin, citrate, etc.) before removal of blood cells by centrifugation [1]. There are no virtual interferences that can occur in serum owing to postcentrifugal coagulation [9,10]. Nevertheless, the presence of anticoagulants in plasma collection tubes can introduce some interferents, such as enzyme inhibitors, fibrinogen and cations [11]. The coagulation procedure creates some differences in the composition of metabolites

Abbreviations: BSTFA, bis-(trimethylsilyl) fluoroacetamide; BPCs, base peak chromatograms; EI, electron impact; ES, external standard; GC, gas chromatography; MF, molecular feature; MPP, mass profiler professional, principal component analysis, quality control; MS, mass spectrometry; MS/MS, tandem mass spectrometry; TOF, time-of-flight; PFTBA, perfluorotri-n-butylamine; RI, retention index; RSD, relative standard deviation; TMCS, trimethylchlorosilane; UPLC, ultra-high-performance liquid chromatography

* Corresponding author at: Postal address: Department of Analytical Chemistry, University of Córdoba, Annex C3, Campus of Rabanales, Córdoba, Spain.
E-mail address: q72prcaf@uco.es (F. Priego-Capote).

http://dx.doi.org/10.1016/j.talanta.2015.12.079
0039-9140/

between the two biofluids, such as in the levels of inflammatory markers. Thus, serum samples are preferred in assays to determine cardiac troponins, whereas plasma is recommended in glucose tolerance tests [1].

The impact of blood sample collection procedures on variations in the concentration of metabolites present in plasma or serum has been tested in several clinical studies [5,7,12–19]. Thus, Jorgenrud et al. (2015) studied the influence of three collection tubes (the conventional tube for serum and those containing citrate or EDTA for plasma) on the stability of some metabolites identified by UHPLC–QTOF MS/MS and GC × GC–TOF/MS [17]. Also, Barri and Dargsted (2013) searched for differences in plasma collected in different tubes (with EDTA, citrate or heparin) and the conventional tube for serum by using untargeted analysis based on UPLC–QTOF MS/MS [7]. However, this study did not consider the inter-individual variability that could mask the real behavior of certain metabolites. Wedge et al. (2011) considered the inter-individual variability, but their study was focused on the differences between plasma and serum samples of patients with small-cell lung cancer by using untargeted analysis based on UPLC–MS and GC–QTOF [14]. Breier et al. (2014) reported metabolite measurements generally higher in serum as compared to plasma for saturated acylcarnitines, amino acids, biogenic amines, glycerophospholipids, sphingolipids and hexose. Additionally, the study was targeted at stability conditions finding the majority of metabolites stable for 24 h both on cool packs and at room temperature in non-centrifuged tubes [18].

There are several types of commercial tubes (tube wall, stopper, stopper lubricant, separator gel, clot activator, etc. [16] for collection of plasma or serum whose composition establishes the subsequent applicability of the collected sample. Among them, it is worth mentioning tubes containing a polymeric gel, an inert material that forms a barrier between the target sample (plasma or serum) and the rest of blood (packed cells) by centrifugation. The main benefits of separator gel tubes are an easy use, short processing time through clot activation, higher yield in the isolation of serum or plasma, reduced aerosolization of hazardous substances and a single centrifugation step [17]. On the other hand, some limitations of these tubes have also been described in the case of hydrophobic drugs such as phenytoin, phenobarbital, carbamazepine, quinidine and lidocaine, which could be adsorbed to the separator gel [17,20]. These tubes have been scarcely studied to assess their applicability in metabolomics analysis by comparison with the use of conventional tubes for serum or plasma. Breier et al. analyzed serum samples collected into conventional and gel-barrier tubes and found that serum metabolite concentrations were mostly unaffected by tube type, except for methionine sulfoxide that was significantly more concentrated in serum collected with gel-barrier tubes [18].

The objective of the present research was to study the differences at the metabolite level between serum and plasma obtained with conventional tubes (heparin tube for plasma) and polymeric gel tubes by application of an untargeted approach based on GC–TOF/MS analysis. For this purpose, a cohort of volunteers was selected for blood sampling, then collected using four different tubes (plasma, plasma-gel, serum and serum-gel). Sample preparation was based on protein precipitation with an organic solvent followed by silylation as derivatization procedure. The differences between the two main clinical samples, plasma and serum, and those ascribed to their collection in polymer gel tubes were discussed taking into account the sources of intra-individual and inter-individuals variability.

2. Materials and methods

2.1. Reagents

Both MS-grade *n*-hexane from Sigma–Aldrich (St. Louis, MO, USA) and methanol from Scharlab (Barcelona, Spain) were used for sample preparation. Bis-(trimethylsilyl) fluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) from Sigma–Aldrich were used as silylation agents in the derivatization step. Pyridine from Merck (Darmstadt, Germany) was used as solvent for derivatization. Mass spectrometry grade perfluorotri-*n*-butylamine (PFTBA) from Agilent Technologies (Santa Clara, CA, USA) was used for daily mass calibration. An alkane standard mixture (from C10 to C40) designed for performance test in GC from Sigma–Aldrich was used to establish the retention index (RI) calibration. Triphenyl phospate (TPP, from Sigma–Aldrich) was used as external standard (ES) and standards of D-mannitol, D-fructose, D-sorbitol, myo-inositol, D-glucose and D-mannose were purchased to confirm identification of sugars.

2.2. Apparatus and instruments

A micro-centrifuge Sorvall Legend Micro 21R from Thermo Scientific (Waltham, MA, US) was used to separate the phases after extraction and protein precipitation. A speed-vac Concentrator Plus, from Eppendorf Ibérica (Madrid, Spain), was used to evaporate the methanol phase before derivatization. A block heater from Stuart Equipment (Staffordshire, OKA, USA) was used in the derivatization step.

An Agilent 7890A Series GC system coupled to an Agilent 7200 UHD Accurate-Mass QTOF hybrid mass spectrometer equipped with an electron impact (EI) source (Santa Clara, CA, USA) was used for analysis. The analytical sample was thus monitored in high resolution mode. MassHunter GC QTOF Acquisition software (version B.06, Agilent Technologies) was used to control data acquisition and set the parameters for optimum operation.

2.3. Samples

All experiments were carried out in accordance with the ethical principles of human medical research (World Medical Association, Helsinki Declaration, 2004 [21]). The ethical review board of Reina Sofía University Hospital (Córdoba, Spain) approved and supervised the clinical study.

The samples were obtained from thirteen healthy volunteers (3 men and 10 women) at Reina Sofía University Hospital (Córdoba, Spain). The steps from blood extraction to analysis were supervised by specialized personnel pertaining to this hospital. Blood from each volunteer was collected into four different Vacutainer® tubes (Becton Dickinson): plastic serum tubes with spray-coated silica (serum), plastic serum tubes with spray-coated silica and a polymer gel to favor serum separation (serum-gel), spray-coated silica tubes with heparin for plasma (plasma) and heparin tubes with polymer gel to favor plasma separation (plasma-gel).

The collected samples were processed by centrifugation for 15 min at 1000 × *g* for isolation of serum and plasma in conventional tubes, and at 2000 × *g* for gel separation tubes. The serum fraction was processed 1 h after collection to ensure complete coagulation.

2.4. Untargeted analysis

Plasma and serum samples (50 µL) were deproteinized with 150 µL of methanol. The mixture was vortexed for 1 min and subsequently cooled at −20 °C for 3 min. The resulting precipitate