Analytica Chimica Acta 951 (2017) 99–107

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Impact of storage conditions on the urinary metabolomics fingerprint

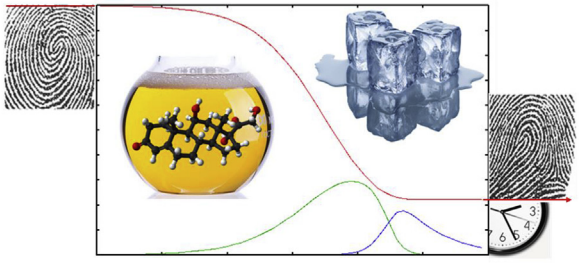
Jérôme Laparre [a, 1], Zied Kaabia [a, 1], Mark Mooney [b], Tom Buckley [c], Mark Sherry [d], Bruno Le Bizec [a], Gaud Dervilly-Pinel [a, *]

[a] LUNAM Université, Oniris, Laboratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, F-44307, France
[b] School of Biological Sciences, Queen's University Belfast (QUB), Medical Biology Centre, 97 Lisburn Road, Belfast, BT9 7BL, United Kingdom
[c] Irish Equine Center, Johnstown, Naas, County Kildare, W91 RH93, Ireland
[d] IDLS Irish Equine Centre, Johnstown, Naas, County Kildare, W91 RH93, Ireland

HIGHLIGHTS

- HILIC and RPLC chromatographies were used for an exhaustive metabolomic approach.
- A chemical database was created (n > 200) to identify affected urine metabolites.
- Urine metabolic profile is altered starting from 5 days following storage at +4C°.
- A number of urine metabolites profiles start being altered after a month at −20C°.
- −80 °C was considered as the most convenient urine long-term storage condition.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form
22 November 2016
Accepted 25 November 2016
Available online 2 December 2016

Keywords:
Urine
Storage
Stability
Metabolite profiling
Metabolites data base

ABSTRACT

Urine stability during storage is essential in metabolomics to avoid misleading conclusions or erroneous interpretations. Facing the lack of comprehensive studies on urine metabolome stability, the present work performed a follow-up of potential modifications in urinary chemical profile using LC-HRMS on the basis of two parameters: the storage temperature (+4 °C, −20 °C, −80 °C and freeze-dried stored at −80 °C) and the storage duration (5–144 days). Both HILIC and RP chromatographies have been implemented in order to globally monitor the urinary metabolome. Using an original data processing associated to univariate and multivariate data analysis, our study confirms that chemical profiles of urine samples stored at +4 °C are very rapidly modified, as observed for instance for compounds such as:*N*-acetyl Glycine, Adenosine, 4-Amino benzoic acid, *N*-Amino diglycine, creatine, glucuronic acid, 3-hydroxy-benzoic acid, pyridoxal, L-pyroglutamic acid, shikimic acid, succinic acid, thymidine, trigonelline and valeryl-carnitine, while it also demonstrates that urine samples stored at −20 °C exhibit a global stability over a long period with no major modifications compared to −80 °C condition. This study is the first to investigate long term stability of urine samples and report potential modifications in the urinary metabolome, using both targeted approach monitoring individually a large number (n > 200) of urinary metabolites and an untargeted strategy enabling assessing for global impact of storage conditions.

* Corresponding author.
E-mail address: gaud.dervilly@oniris-nantes.fr (G. Dervilly-Pinel).
[1] Both authors contributed equally.

http://dx.doi.org/10.1016/j.aca.2016.11.055
0003-2670/

1. Introduction

Metabolomics, which has become a cornerstone in the field of systems biology research, consists of the large scale and high-throughput measurement of the global "metabolome", comprised of low molecular-weight metabolites (<1500 Da), in biological matrices [1,2]. The main analytical aim of such an approach is to investigate potential differences in the chemical signature of biological samples that are representative of changes in the physiology of the system(s) under study. Subsequently, the objective resides in the identification of those metabolites or metabolic pathways associated with these observed differences. Such chemical signatures can be the consequence of disease, drugs, dietary, environmental exposition, etc. In this regard, urine metabolomics has recently emerged as a promising arena for the discovery of non-invasive biomarkers signifying metabolic disruptions in response to specific diseases or exposures. Due to the reduced intricacy of sample pre-treatment, lower protein content and sample complexity including reduced molecular interaction, and combined with simple and non-invasive collection, urine possesses a number of advantages as a test matrix and is therefore deemed highly amenable from a metabolomics context. Indeed, compared to other biofluids, urine is characterized by its ease of collection, richness in metabolites and its ability to reflect imbalances of all biochemical pathways within the body [3,4]. Within the global profile reflecting metabolic alteration, it is expected that most of the differences observed in the chemical signatures is induced as a result of the studied effect, but it may also happen that it simply arises from occurring biologically (both inter- and intra-individual) expressed variations [5], therefore requiring extended knowledge and control of the systems under study to prevent any misleading conclusions. Even less desirable are those analytical pitfalls occurring during the metabolomics workflow which may be responsible for the generation of chemical variations in the metabolomics fingerprint leading also to potentially erroneous biological interpretation of the observations. Consequently, the control and minimization of potential sources of variability exposed above is crucial to avoid errors in data interpretation. Following inter- and intra-individual variations, the major source of error in metabolomics is principally associated with sampling and post-collection procedures (e.g., freeze-thaw cycles or inadequate storage conditions [6,7]), and it is recognized that unsuitable sampling and sample pretreatment protocols can lead to biased results due to conversion or degradation of metabolites [7,8]. Therefore, the non-targeted aspect of metabolomics approaches, consisting by definition in the monitoring of "unknown" compounds, should not be hampered by such confounding factors that arise at the different stages of the metabolomics workflow, and specifically during the biological sample handling steps. In particular, the variability associated with sample storage is considered critical to metabolomics approaches due to the intrinsic biochemical activity of biological matrices, and particular care should be granted to this step since the stability of many metabolites is strongly affected by associated parameters, mainly time and temperature. Hence, these conditions may impact upon the chemical fingerprints generated, modifying them leading to potential misinterpretation and erroneous association to the parameter(s) of interest investigated within the given study. Metabolic activity during sampling and storage therefore requires eliminating or minimizing any possible changes in the metabolic profile, both from a qualitative and quantitative aspect. For this purpose, reduced temperatures during sample storage (+4 °C or at/below −20 °C) are common strategies [9,10], and it is widely accepted that most metabolites are preserved if samples are immediately frozen [4,11].

The importance of these pre-analytical aspects to the performance of metabolomics studies and particularly the critical issues associated with urine storage conditions have previously been reviewed [7,12,13]. However, since there is a demand for the development of robust strategies for preparation of biological samples that can be reproduced across different laboratories within the scientific community, efforts must be made to standardize collection and storage conditions prior to conduction of metabolomics studies *per se* [7,14]. The need for validation processes in the clinical and pharmaceutical fields has also led to extensive review of the pitfalls associated with preliminary sample preparative steps [15,16]. The investigation of the impact of sample storage conditions on the urine metabolome has principally been reported based on NMR [6,9,17] which previously was adopted as the technique of choice [18] due to a minimal sample preparation prior to analysis and high throughput capacity. Such studies revealed a limited impact of storage temperature conditions of −20 °C or lower on the chemical signature obtained over time [9,17]. As an example, Lauridsen et al. [6] found that human urine samples could be stored at or below −25 °C for 26 weeks without changes to acquired 1H NMR fingerprints. However, in comparison to what are now more commonly used fingerprinting techniques such as mass spectrometry (MS), NMR is limited in terms of detection sensitivity and is laboriously compatible with up-stream dimensions of separation such as chromatography, that provides access to an extended dynamic range of detectable compounds [19]. In this context, more recent studies have investigated the impact of urine storage on the metabolome fingerprint developed using either GC or LC chromatography coupled to MS. Whilst some of these studies have examined the short-term (one or few days) effect of +4 °C [13] storage conditions on urinary metabolite profiles, others have focused on long-term storage effects [12]. No significant modifications to the urinary fingerprints could be highlighted either at −20 °C or −80 °C [12,20], which may explain why most studies related to the investigation of the urinary metabolome have for practical reasons used −20 °C storage conditions [10]. It is however worth noting that differences in storage time or frequent thaw/freeze cycles may have a strong influence on the development of metabolomics models [7].

The lack of available comprehensive studies on urine metabolome stability has recently been highlighted [4] and the present study therefore proposes to perform a follow-up of potential modifications to urinary metabolic profiles using LC-HRMS based on the effects of two parameters: storage temperature (+4 °C, −20 °C, −80 °C and freeze-dried stored at −80 °C) and storage duration (5–144 days). Both HILIC and RP chromatographies have been implemented in order to monitor the global urinary metabolome. Following LC-HRMS acquisition, an original data processing step was developed and applied in order to extract biologically and analytically relevant variables. Complementary multivariate and univariate analysis were performed on the acquired datasets after normalization in order to highlight the sample metabolic profile alteration dependent on sample storage conditions and duration. Univariate analysis were processed both on the global set of "unknown" extracted variables and on a set of determined variables (n = 115) previously identified on the basis of a home-made chemical database (n = 211). No preservatives were added to the urine samples which had previously been filtered to remove bacterial presence and interference. Storage at −80 °C was considered in the present study to be the condition offering greatest stability and it was hypothesized that metabolites investigated through metabolomics profiling were stable over 5 months under such conditions [21]. With regard to the impossibility to have a chemically identical fresh urine sample over 144 consecutive days, urine samples stored at −80 °C were consequently considered