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Optimization study for metabolomics analysis of human sweat by liquid chromatography–tandem mass spectrometry in high resolution mode

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ABSTRACT

Sweat has recently gained popularity as a potential tool for diagnostics and biomarker monitoring as it is a non-invasive biofluid the composition of which could be modified by certain pathologies, as is the case with cystic fibrosis, which increases chloride levels in sweat. The aim of the present study was to develop an analytical method for analysis of human sweat by liquid chromatography–mass spectrometry (LC–Q-TOF MS/MS) in high resolution mode. Thus, different sample preparation strategies and different chromatographic modes (HILIC and C18 reverse modes) were compared to check their effect on the profile of sweat metabolites. Forty-one compounds were identified by the MS/MS information obtained with a mass tolerance window below 4 ppm. Amino acids, dicarboxylic acids and other interesting metabolites such as inosine, choline, uric acid and tyramine were identified. Among the tested protocols, direct analysis after dilution was a suited option to obtain a representative snapshot of sweat metabolome. In addition, sample clean up by C18 SpinColumn SPE cartridges improved the sensitivity of most identified compounds and reduced the number of interferents. As most of the identified metabolites are involved in key biochemical pathways, this study opens new possibilities to the use of sweat as a source of metabolite biomarkers of specific disorders.

1. Introduction

Sweat is an aqueous electrolyte solution excreted by the eccrine and apocrine sweat glands originated in the skin dermis of mammals and terminated in the secretory canals that flow into the skin surface and hair follicles. The primary function of sweating is thermoregulation to control body temperature by evaporative cooling. In addition, sweat is a defence mechanism of skin, the excretion fluid of chemosignals such as androstadienone – which acts as hormonal stimuli in females –, and waste of metabolites such as uric acid [1]. Sweat is mainly composed by water, but it contains several minor components including electrolytes, ammonia, urea, small molecules such as carboxylic acids and amino acids as well as more complex biomolecules such as proteolytic enzymes and antimicrobial peptides, among others [2]. Therefore, the varied composition of sweat supports its clinical interest to be potentially exploited for diagnostic.

The scant application of sweat in clinical analysis is explained by the lack of studies to relate sweat composition and pathological states. In fact, few clinical tests use sweat samples. One of these tests is for diagnosis of cystic fibrosis in new-borns based on determination of chloride in sweat [3–5]. However, the advances both in sweat collection devices and sensitive analytical techniques increased the interest on sweat testing of drugs over the past few years [6–10]. Sweat may offer a non-invasive alternative to present sampling for continuous monitoring of drugs exposure as sweat can be collected for a programmed period with minimal disturbance for the sampled individual. Sweat sampling can be performed with the aid of sweat wipes as liquid perspiration or over time using sweat patches [11]. The experience gained in sweat collection procedures for the analysis of xenobiotics could be used to implement this biofluid in clinical diagnostics [12,13].

Major limitations in the analysis of sweat are the low volume of sweat secreted and the variability in secretion among individuals [14,15]. Also, sampling is a complex process since the collected sweat should be representative and unaffected by interferents.

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Non-invasiveness and the absence of requirements in terms of health personnel care are the main benefits from sweat as clinical sample.

Despite the scant studies on the clinical perspectives of sweat, the potential of this biofluid in omics disciplines has been pointed out. In proteomics, a current research developed by Raiszadeh et al. revealed that sweat proteome is rather different from serum proteome [16]. Therefore, human sweat could be considered an additional source of unique disease-associated biomolecules. In fact, Raiszadeh et al. found differential abundances of selected proteins between schizophrenia patients and control individuals. This preliminary test should be validated to prove the applicability of human sweat in the diagnostic of schizophrenia. In the metabolomics field, a recent study of sweat by high-resolution NMR spectroscopy has revealed that some of the components present in human sweat are involved in primary and secondary biological functions [17]. Among them, amino acids, sugars, lactate, glycerol, and compounds involved in the citric acid cycle (e.g. pyruvate, fumarate or aconitate) have been detected. NMR is especially suited to metabolomics profiling of human sweat as this is a relatively non-complex biofluid. Nevertheless, more research on sweat composition is demanded to assess the potential of this biofluid for clinical diagnostic. The aim of the present study was to develop a method for analysis of human sweat by LC–Q-TOF MS/MS. Different sample preparation strategies such as sample dilution, acid/alkaline hydrolysis and solid-phase extraction (SPE) were compared to check their influence on the profile of the detected metabolites. Taking into account the polar character of human sweat, two analytical chromatographic columns, C18 and HILIC, were tested to study their suitability to analyze human sweat. Identification of metabolites by LC–Q-TOF MS/MS in high resolution mode was carried out to obtain a snapshot of the composition of sweat metabolome.

2. Experimental

2.1. Reagents

MS-grade formic acid, ammonium formate and acetonitrile (ACN) to prepare the chromatographic mobile phases and sodium hydroxide and hydrochloric acid were purchased from Scharlab (Barcelona, Spain). Deionized water (18 mΩ cm) from a Millipore Milli-Q water purification system was used.

2.2. Instruments and apparatus

An Agilent 1200 Series LC system (consisting of a binary pump, a vacuum degasser, an autosampler and a thermostated column compartment) coupled to an Agilent 6540 UHD Accurate-Mass Q-TOF hybrid mass spectrometer equipped with dual electrospray ionization (ESI) source (Santa Clara, CA, USA) was used. The chromatographic eluate was thus monitored in high resolution mode.

2.3. Cohort selected for the study

A cohort of 96 individuals with an average age of 59 ± 11 years and a proportion of 75% male individuals participated in this study. All steps from sweat sampling to analysis were performed in compliance with the guidelines dictated by the World Medical Association Declaration of Helsinki of 2004. The study was approved by the ethics committee of the Reina Sofia University Hospital. The individuals selected for this study were previously informed to obtain consent.

2.4. Sweat producer and collector: procedure

All samples were collected from 9 to 11 am after breakfast ingestion to fix the most common conditions of clinical practice. A Macroduct® Sweat Analysis System (Wescor, UT, USA), consisting of a Webster sweat inducer and a Macroduct sweat collector (US Patent 4,542,751), was used. Pilogel® iontophoretic disks (US Patent 4,383,529) (Wescor, UT, USA), a gel reservoir of pilocarpinium ions, were used in the iontophoretic stimulation of sweat.

The sweat inducer provided a current intensity of 1.5 mA for 5 min through two pilogel disks as electrodes located on the forearm. After removing the disks the skin where the positive disk had been located was cleaned with distilled water and the Macroduct collector covered this skin to collect sweat for 15 min. The collected sweat was transferred to a micro Eppendorf and stored at −80 °C until use.

One individual was randomly selected to collect seven sweat samples in different days which were used to study sample variability and methodological reproducibility.

2.5. Sample treatment

A pool was prepared taking 5 μL of samples from all participants. Then, different experimental protocols were compared to select that providing maximum metabolite coverage. Firstly, 10 μL of sample was diluted with 20 μL of 0.1% (v/v) formic acid in water to analyze directly sweat without treatment. Then, two different strategies were tested.

(i) Sweat hydrolysis under acid or alkaline conditions to release metabolites conjugated to proteins or peptides. With this aim, 100 μL aliquots were 1:1 mixed with 0.1 M either NaOH or HCl in water and vortexed at room temperature for 30 min, then evaporated to dryness and reconstituted by 100 μL of chromatographic mobile phase A.
(ii) Sweat clean up and preconcentration by solid phase extraction using C18 and hydrophilic centrifugal Micro SpinColumn™ systems (Harvard Apparatus, MA, USA) and following the protocol recommended by the manufacturer depending on the sorbent material.

The protocol for C18 Micro SpinColumn™ was as follows: 150 μL of water for solvation, 150 μL of 50% (v/v) acetonitrile for sorbent conditioning, 150 μL of 5% (v/v) acetonitrile for sorbent equilibration, 25 μL of sample, 150 μL of 5% (v/v) acetonitrile to obtain the sample free from the undesirable retained compounds, which were subsequently eluted by 150 μL of 50% (v/v) acetonitrile. Centrifugation for 25 s at 1000 × *g* was used for each step.

The protocol for hydrophilic Micro SpinColumn™ system was: 150 μL of water for solvation, 150 μL of 50% (v/v) acetonitrile for sorbent conditioning, 150 μL of 5% (v/v) acetonitrile for sorbent equilibration, 25 μL of sample, 150 μL of acetonitrile to remove undesired non-retained compounds, and 150 μL of 50% (v/v) acetonitrile to elute the retained target compounds. The centrifugation conditions were as those used for the C18 sorbent.

Both the eluate and the non-retained fraction (collected after sample application) obtained in each SPE protocol were analyzed separately after evaporation and reconstitution by 20 μL of water acidified with 0.1% formic acid.

2.6. LC–Q-TOF MS/MS analysis

Two chromatographic columns were tested for separation. A C18 reverse-phase analytical column (Mediterranean, 50 mm × 0.46 mm i.d., 3 μm particle size) from Teknokroma