



ELSEVIER

Contents lists available at ScienceDirect

MethodsX

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

Method Article

Metabolic profiling of body fluids and multivariate data analysis



Jean-Pierre Trezzi^{a,b}, Christian Jäger^a, Sara Galozzi^c,
Katalin Barkovits^c, Katrin Marcus^c, Brit Mollenhauer^{d,e},
Karsten Hiller^{a,f,g,*}

^a Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belvaux, Luxembourg

^b Integrated BioBank of Luxembourg, Strassen, Luxembourg

^c Functional Proteomics, Medizinisches Proteom-Center, Ruhr-University Bochum, Germany

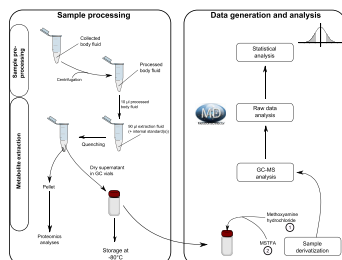
^d Paracelsus-Elena Klinik, Kassel, Germany

^e University Medical Center Goettingen, Institute of Neuropathology and Department of Neurosurgery, Goettingen, Germany

^f Braunschweig Integrated Centre of Systems Biology, University of Braunschweig, Rebenring 56, Braunschweig, Germany

^g Department of Computational Biology of Infection Research, Helmholtz Centre for Infection Research, Braunschweig, Germany

GRAPHICAL ABSTRACT



Quantitative multi-component analysis of body fluids with GC–MS. After sample collection, the samples are pre-processed and used for metabolite extraction. After this, GC–MS analysis and subsequent data processing and analysis are performed.

* Corresponding author at: Braunschweig Integrated Centre of Systems Biology, University of Braunschweig, Rebenring 56, Braunschweig, Germany.

E-mail address: karsten.hiller@tu-braunschweig.de (K. Hiller).

A B S T R A C T

Metabolome analyses of body fluids are challenging due pre-analytical variations, such as pre-processing delay and temperature, and constant dynamical changes of biochemical processes within the samples. Therefore, proper sample handling starting from the time of collection up to the analysis is crucial to obtain high quality samples and reproducible results. A metabolomics analysis is divided into 4 main steps: 1) Sample collection, 2) Metabolite extraction, 3) Data acquisition and 4) Data analysis.

Here, we describe a protocol for gas chromatography coupled to mass spectrometry (GC–MS) based metabolic analysis for biological matrices, especially body fluids. This protocol can be applied on blood serum/plasma, saliva and cerebrospinal fluid (CSF) samples of humans and other vertebrates. It covers sample collection, sample pre-processing, metabolite extraction, GC–MS measurement and guidelines for the subsequent data analysis.

Advantages of this protocol include:

- Robust and reproducible metabolomics results, taking into account pre-analytical variations that may occur during the sampling process
- Small sample volume required
- Rapid and cost-effective processing of biological samples
- Logistic regression based determination of biomarker signatures for in-depth data analysis

© 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

A R T I C L E I N F O

Method name: Metabolic profiling of body fluids and multivariate data analysis

Keywords: Metabolomics, Metabolite extraction, Blood, Saliva, Cerebrospinal fluid, GC–MS, Data analysis, Body fluids

Article history: Received 9 May 2016; Accepted 10 February 2017; Available online 20 February 2017

Method details*Materials*

- 50 μ L body fluid (for analysis in triplicates), such as plasma, serum, saliva or CSF (fresh or stored at -80°C)
- Methanol (High purity, LC–MS grade) at -20°C
- Internal standard [$\text{U-}^{13}\text{C}$]Ribitol (Omicron Biochemicals, ALD-062)
- Sample collection tubes, such as sterile collection tubes (for CSF and saliva), EDTA and serum-separating tubes (for blood)
- Wet ice
- Methoxyamine hydrochloride 98% (Sigma-Aldrich, 226904)
- Pyridine 99.8% (Sigma-Aldrich, 270970)
- N-methyl-N-trimethylsilyl-trifluoroacetamide (Macherey-Nagel, 701270.110)
- Alkane standard mixture for performance tests of GC-systems (Sigma-Aldrich, 68281-10ml-F)
- GC glass vials with micro insert (gastight) 5–250 μ L (various suppliers)
- (Magnetic) caps for GC glass vials (various suppliers)

All aqueous solutions used throughout this protocol should be prepared with MilliQ or deionized water (18.2 $\text{M}\Omega$ cm, <3 ppb TOC).

Note: The proper selection of the collection tubes is highly relevant for GC–MS based metabolomics analyses. In Section Choice of collection tubes we review and recommend several types of collection tubes for the different body fluid types. In general, we recommend the use of sterile tubes to reduce the risk of sample contamination.

Equipment

- Reaction tube centrifuge, such as Eppendorf 5424R
- Refrigerated rotary vacuum evaporator, such as CentriVap Concentrator refrigerated (Labconco)

Download English Version:

<https://daneshyari.com/en/article/5518455>

Download Persian Version:

<https://daneshyari.com/article/5518455>

[Daneshyari.com](https://daneshyari.com)