Journal of Chromatography A, 1464 (2016) 21–31

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Development and validation of a high-throughput micro solid-phase extraction method coupled with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry for rapid identification and quantification of phenolic metabolites in human plasma and urine

Rodrigo P. Feliciano [a], Elsa Mecha [b], Maria R. Bronze [b,c,d], Ana Rodriguez-Mateos [a,*]

[a] *Division of Cardiology, Pulmonology and Vascular Medicine, University of Düsseldorf, Universitätsstraße 1, Düsseldorf 40225, Germany*
[b] *Instituto de Tecnologia Química e Biológica (ITQB), Universidade Nova de Lisboa, Oeiras, Portugal*
[c] *Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal*
[d] *iMED, Faculdade de Farmácia, Universidade de Lisboa, Av. das Forças Armadas, 1649-019 Lisboa, Portugal*

ARTICLE INFO

Article history:
Received 30 May 2016
Received in revised form 10 August 2016
Accepted 11 August 2016
Available online 11 August 2016

Keywords:
Method validation
UHPLC Q-TOF-MS
Micro solid-phase extraction
(Poly)phenols
Plasma
Urine

ABSTRACT

A rapid and high-throughput micro-solid phase extraction (μ-SPE) method coupled with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC Q-TOF MS) analysis was optimized and validated for the quantification of 67 (poly)phenol metabolites in human plasma and urine using authentic standards. The method was fully validated in terms of specificity, linearity, method detection limit (MDL), method quantification limit (MQL), repeatability, intra- and inter-day precision, accuracy and matrix effects. The method proved to be specific and results showed linearity of responses for all compounds, with MDL ranging between 0.04 nM and 86 nM in plasma and between 0.01 nM and 136 nM in urine. MQL ranged between 0.14 nM and 286 nM in plasma and between 0.03 nM and 465 nM in urine. Repeatability varied between 1.7 and 9.2% in plasma and between 2.2% and 10.4% in urine. Median precision values of 8.7 and 11.5% (intra-day), and 10.8% and 10.0% (inter-day) were obtained in plasma and urine, respectively. The median recovery was 89% in both biological matrices. Matrix effects were determined and median values of −1.2% and −6.8% in plasma and urine were obtained. After method validation, 49 and 57 compounds, including phase II and gut microbial metabolites, were quantified in plasma and urine, respectively, following cranberry juice consumption. This methodology can be applied to large-scale human dietary intervention trials allowing for high sample throughput.

© 2016 Published by Elsevier B.V.

1. Introduction

The consumption of (poly)phenol-rich foods such as fruits and vegetables has been associated with several benefits in human health, including prevention of cardiovascular disease and cancer [1–4]. Once ingested, (poly)phenols are extensively metabolized by phase I and II enzymes after absorption in the stomach and small intestine, yielding mostly methylated, sulfated and glucuronidated metabolites [5]. A large amount of the ingested polyphenols are not absorbed in the small intestine and reach the colon intact, where they are metabolized by the gut microbiota before absorption [6]. Generally, parent compounds are detected in much lower concentrations in plasma and urine [7] than their metabolites [8]. For example, only 1% of (−)-epicatechin has been found intact in plasma while 99% corresponded to phase II metabolites [9]. Therefore, sensitive methodology is needed to quantify potentially bioactive metabolites in biofluids since they can account, at least, partially, for the benefits seen after consumption of (poly)phenol-rich foods. Furthermore, in order to correlate clinical health effects with the appearance of (poly)phenol metabolites in circulation it is crucial to implement robust analytical methods which can accurately quantify these compounds. This is also important for the development of biomarkers of (poly)phenol intake in epidemiological studies.

As many analytical standards of (poly)phenol metabolites, in particular phase II metabolites, are not commercially available,

* Corresponding author.
E-mail addresses: anarodriguezmateos@gmail.com, guriagalega@yahoo.es (A. Rodriguez-Mateos).

http://dx.doi.org/10.1016/j.chroma.2016.08.027
0021-9673/© 2016 Published by Elsevier B.V.

most of the past work have used enzymatic hydrolysis with glucuronidase and sulfatase to estimate concentrations found *in vivo* [10,11], or used the parent compounds to quantify phase II metabolites [12,13]. However, both approaches have significant drawbacks, such as incomplete hydrolysis, different activity and purity of commercially available enzymes [14,15], or higher inaccuracy due to the differences in responses between aglycone and conjugated compounds [16,17]. The best alternative is to synthesize compounds which are not available, as it has been recently reported for valerolactones, and flavonoid sulfates and glucuronides [18–20]. To date, very few validated methods exist that used authentic phase II metabolite standards for quantification, and those methods focused on anthocyanin or ellagic acid metabolites [16,21], limiting their applicability to other polyphenol classes. Importantly, sample preparation in those methods is not adequate for high-throughput analysis, which is essential for large scale epidemiological and clinical studies.

Micro-elution solid-phase extraction (μ-SPE) provides an excellent option for analysis of (poly)phenol metabolites since it requires small sample volumes [22,23], it is fast and the elution can be semi-automated using a positive pressure 96-well plates device. By combining μ-SPE with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC Q-TOF MS) we were able to develop and validate a very fast high-throughput method to quantify (poly)phenol metabolites in plasma and urine. After method validation, concentration of (poly)phenol metabolites in plasma and amounts excreted in urine were determined after intake of a single-strength cranberry juice by ten healthy young volunteers. As far as we know, this is the first report on the validation of an analytical method which is able to quantify as many as 67 (poly)phenol metabolites in urine and plasma using authentic standards, with little sample preparation and an analytical run time of only 10 min.

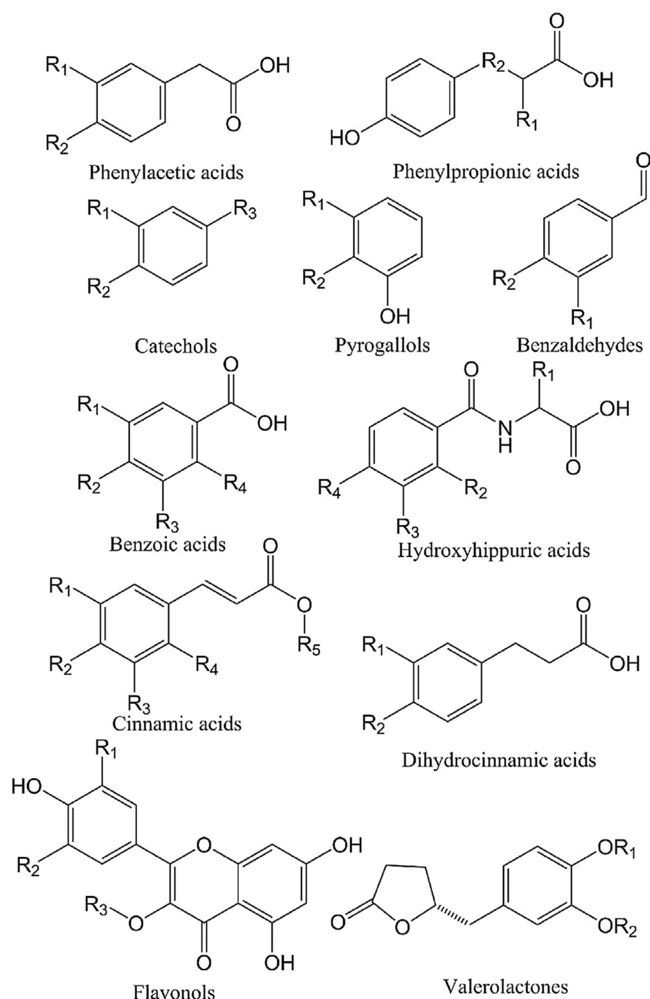

Fig. 1. Structure of the main (poly)phenol class of compounds used for the validation study. Substituents (R_{1-5}) can correspond to −H, $-CH_2$, $-CH_3$, −O, −OH, $-OCH_3$, $-OSO_3H$, −*O*-glucuronide or quinic acid. Detailed structures for the 67 compounds used in this study can be found in Supplementary information.

2. Materials and methods

2.1. Materials and reagents

Homovanillic acid sulfate sodium salt, caffeic acid 3-*O*-β-D-glucuronide, caffeic acid 4-*O*-β-D-glucuronide, dihydrocaffeic acid 3-*O*-sulfate sodium salt, dihydrocaffeic acid 3-*O*-β-D glucuronide diammonium salt, ferulic acid 4-*O*-β-D-glucuronide disodium salt, ferulic acid 4-*O*-sulfate disodium salt, dihydroferulic acid 4-*O*-sulfate sodium salt, dihydroferulic acid 4-*O*-β-D-glucuronide, isoferulic acid 3-*O*-sulfate disodium salt, isoferulic acid 3-*O*-β-D-glucuronide, dihydro isoferulic acid 3-*O*-sulfate disodium salt, dihydro isoferulic acid 3-*O*-ß-D-glucuronide and (4*R*)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone-4′-*O*-sulfate sodium salt were obtained from Toronto Research Chemicals (Toronto, Canada). The valerolactone was provided without information regarding the exact position of the sulfate and will be designated in this work as (4*R*)-5-(3′-hydroxyphenyl)-γ-valerolactone-4′-*O*-sulfate, according to the NMR spectrum provided by the supplier. Kaempferol-3-*O*-ß-D-glucuronide was obtained from Extrasynthese (Genay, France). 1-Methylpyrogallol-*O*-sulfate, 2-methylpyrogallol-*O*-sulfate, 4-methylcatechol-*O*-sulfate, 4-methylgallic-3-*O*-sulfate, catechol-*O*-sulfate, pyrogallol-*O*-1-sulfate, pyrogallol-*O*-2-sulfate and vanillic acid-4-*O*-sulfate were kindly provided by Dr Cláudia Nunes dos Santos and Dr Rita Ventura, and their synthesis has been described elsewhere [24]. 2-, 3- and 4-hydroxyhippuric acids were purchased from Enamine (Kiev, Ukraine). All the polyphenol and phenolic acid aglycones were obtained from Sigma-Aldrich Co. (Steinheim, Germany). Eleven different classes of polyphenols were included in this study (Fig. 1) and the complete list of compounds evaluated is presented in Supplementary information (Fig. 1). Acetic acid was from Carl Roth (Karlsruhe, Germany) and OASIS HLB μElution plates (2 mg sorbent per well, 30 μm) were from Waters (Eschborn, Germany). Milli-Q system (Merck KGaA, Darmstadt, Germany) ultrapure water was used. Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Co. (Steinheim, Germany).

2.2. Plasma and urine collection

For the method validation study, blood and urine of four healthy volunteers was collected. These subjects were fasted and had followed a 72 h low-polyphenol diet and no fruits or vegetables were consumed at least 24 h before samples collection. After method validation, ten healthy volunteers followed the same low-polyphenol diet and consumed 450 mL of a single-strength cranberry juice beverage containing 787 mg of (poly)phenols, mainly proanthocyanidins (710 mg) and smaller amounts of flavonols (31.3 mg), phenolic acids (24.5 mg), anthocyanins (16.2 mg), and flavan-3-ols (5.0 mg) [24]. Blood was collected at baseline and at 1, 2, 4, 6 and 8 h post-consumption, whereas urine was collected at baseline and after 8 and 24 h. The details of this study have been previously described [24].

Plasma was obtained by whole-blood centrifugation (EDTA-containing vacutainers) at 1800*g* for 15 min at 4 °C, and spiked

Download English Version:

https://daneshyari.com/en/article/1198781

Download Persian Version:

https://daneshyari.com/article/1198781

Daneshyari.com