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Ultra high performance liquid chromatography-quadrupole-time of flight analysis for the identification and the determination of resveratrol and its metabolites in mouse plasma

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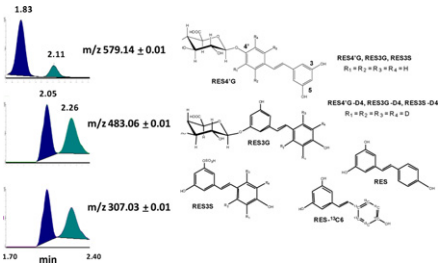
HIGHLIGHTS

- Fast method to quantify resveratrol and its main metabolites in the mouse plasma.
- Isotope-labeled standards to build a linear calibration curve.
- Linear calibration curve on a wide range of concentrations.
- Simultaneous identification and quantification of metabolites by using full scan mode.
- Detection of uncommon metabolites not yet described in mice.

GRAPHICAL ABSTRACT

Simultaneous identification and determination of new resveratrol metabolites in mice by UHPLC-Q-TOF in full scan mode.



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ABSTRACT

Resveratrol is a polyphenol that has numerous interesting biological properties, but, per os, it is quickly metabolized. Some of its metabolites are more concentrated than resveratrol, may have greater biological activities, and may act as a kind of store for resveratrol. Thus, to understand the biological impact of resveratrol on a physiological system, it is crucial to simultaneously analyze resveratrol and its metabolites in plasma. This study presents an analytical method based on UHPLC-Q-TOF mass spectrometry for the quantification of resveratrol and of its most common hydrophilic metabolites. The use of ^{13}C- and D-labeled standards specific to each molecule led to a linear calibration curve on a larger concentration range than described previously. The use of high resolution mass spectrometry in the full scan mode enabled simultaneous identification and quantification of some hydrophilic metabolites not previously described in mice. In addition, UHPLC separation, allowing run times lower than 10 min, can be used in studies that requiring analysis of many samples.

Abbreviations: ACN, acetonitrile; CV, coefficient of variation; DEV, deviation; ESI, electrospray ionization; FA, formic acid; HPLC, high performance liquid chromatography; IS, internal standard; IV, intravenous; LLOQ, lower limit of quantification; MeOH, methanol; MRM, multiple reaction monitoring; MS, mass spectrometry; QC, quality control; SD, standard deviation; SIDA, stable isotope dilution analysis; UHPLC-Q-TOF, ultra high performance liquid chromatography-quadrupole-time of flight; UV, ultraviolet.

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1. Introduction

Resveratrol is a polyphenol which can be found in more than seventy plant species and in particular in grapes. Resveratrol has numerous biological properties: it has shown antioxidant [1], anti-inflammatory [2] and anticancer properties in vitro and in vivo in rodents [3]. It may also protect against cardiovascular diseases [4] and improve disorders of carbohydrate and lipid metabolisms [5]. Last but not least, resveratrol is described as a mimetic agent of the caloric restriction as well as an activator of sirtuins [6].

The first studies of resveratrol metabolism have highlighted that it is quickly absorbed and metabolized at the level of the intestine and finally fast metabolized by the liver. Hence, the concentrations of plasmatic resveratrol are low compared to its main glucuronide and sulfate metabolites [7]. Some authors doubt resveratrol's efficacy, particularly when it is only consumed in small quantities as in wine consumption [8,9] and numerous studies have aimed to improve the bioavailability of free resveratrol [10]. It has been also suggested that its metabolites may also have biological activities [11,12] and thus represent a kind of resveratrol store [13]. Today, more than ten metabolites have been identified in humans [7]. To understand the biological impact of resveratrol, it is crucial to analyze simultaneously resveratrol and its metabolites in plasma.

Numerous analytical methods for resveratrol and its metabolites in plasma have already been described, for a large range of species [14–26]. They involved gas chromatography [24] or liquid chromatography coupled to ultraviolet (UV) detection or mass spectrometry (MS). Because resveratrol and its main hydrophilic metabolites vary widely in their hydrophobicity, the separations by high performance liquid chromatography (HPLC) already described for their determination in biological samples are usually long and last at least 20 min [22]. To decrease this analysis time, some authors such as Muzzio et al. used two different gradients of the mobile phase for the resveratrol and for its metabolites [23]. In these studies [14,16,19,20], detection using MS was generally conducted without specific internal standards such as isotope-labeled analogs with ^{13}C or D for resveratrol and its metabolites. Therefore, they did not permit to compensate the variations of the ionization yield of metabolites due to the matrix effects [27]. Moreover, in general, the quantification methods involving HPLC/MS/MS and described in literature correspond to targeted analyses based on the use of specific multiple reaction monitoring (MRM) transitions [19,20,22]. Then, they do not allow identifying and determining the concentration of unknown metabolites and/or whose specific collision-induced dissociation pathways are not described.

In this study, we developed and validated an original and fast method to quantify *trans*-resveratrol and its main metabolites, i.e. *trans*-resveratrol-4′-O-β-D-glucuronide, *trans*-resveratrol-3-O-β-D-glucuronide and *trans*-resveratrol-3-sulfate in the mouse plasma, thanks to UHPLC-Q-TOF (ultra high performance liquid chromatography-quadrupole-time of flight) mass spectrometry with full scan data acquisition mode and to an internal calibration using ^{13}C-labeled resveratrol and D-labeled standard compounds for the metabolites. Because high resolution mass measurements provided structure identification based on accurate mass, this full scan data acquisition mode enabled to detect other hydrophilic metabolites of *trans*-resveratrol and to quantify them together with the other above mentioned metabolites.

2. Experimental

2.1. Chemicals and materials

Reference standard (Fig. 1) of *trans*-resveratrol (99%, w w^{-1}) was purchased from Sigma (Saint-Quentin-Fallavier, France). *Trans*-resveratrol-$^{13}C_6$ was obtained from LGC Standard (Molsheim, France). Reference standards of *trans*-resveratrol-3-O-β-D-glucuronide, *trans*-resveratrol-4′-O-β-D-glucuronide, *trans*-resveratrol-3-sulfate, *trans*-resveratrol-3-O-β-D-glucuronide-D_4, *trans*-resveratrol-4′-O-β-D-glucuronide-D_4 and *trans*-resveratrol-3-sulfate-D_4 were provided by Spi Bio, Bertin Pharma (Montigny-le-Bretonneux, France). Because of the low stability of resveratrol and its derivatives under UV exposure [28], all reference analytes and internal standards were stored at dark at −20 °C. HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (Nogent-sur-Marne, France). Formic acid (FA, ≥99%, w w^{-1}) of HPLC grade was obtained from Fischer Scientific (Illkirch, France). All other chemicals were of analytical grade, and the solvents used were of chromatographic grade. Water was prepared with a Milli-Q water purification system (Millipore, Molsheim, France). Resveratrol (98.5%, w w^{-1}) used for the gavage of mice was provided by Yvery (Marseille, France).

2.2. Stock solutions of calibration standards and internal standards

Initial standard stock solutions, i.e. 5 mg mL^{-1} of *trans*-resveratrol (2.2×10^{-2} mol L^{-1}), *trans*-resveratrol-4′-O-β-D-glucuronide (1.24×10^{-2} mol L^{-1}), *trans*-resveratrol-3-O-β-D-glucuronide (1.24×10^{-2} mol L^{-1}) and *trans*-resveratrol-3-sulfate (1.63×10^{-2} mol L^{-1}) were prepared in MeOH. All stock solutions of analytes and internal standards were stored at −20 °C in the dark.

For method validation and calibration, different stock solutions that combine all four compounds were then prepared in a mixture ACN/water (20/80; V V^{-1}) at concentrations twenty times higher than those of calibration standards, i.e. 0.5 μmol L^{-1}, 1 μmol L^{-1}, 2 μmol L^{-1}, 4 μmol L^{-1}, 8 μmol L^{-1}, 20 μmol L^{-1}, 40 μmol L^{-1}, 80 μmol L^{-1}, 120 μmol L^{-1}, 160 μmol L^{-1}and 320 μmol L^{-1}. These combined stock solutions were used to prepare the calibration standards and quality control (QC) samples.

The concentrations of the internal standards in the stock solutions, *trans*-resveratrol-$^{13}C_6$, *trans*-resveratrol-4′-O-β-D-glucuronide-D_4, *trans*-resveratrol-3-O-β-D-glucuronide-D_4 and *trans*-resveratrol-3-sulfate-D_4, were 5 mg mL^{-1} in MeOH. The final internal standard stock solution combining all four internal standards with concentrations of 40 μmol L^{-1} each of *trans*-resveratrol-3-sulfate-D_4 and *trans*-resveratrol-$^{13}C_6$ and 200 μmol L^{-1} each of *trans*-resveratrol-4′-O-β-glucuronide-D_4 *and trans*-resveratrol-3-O-β-glucuronide-D_4, was prepared in the mixture ACN/water (20/80; V V^{-1}).

2.3. Animals and sample collection

Swiss mice were obtained from Janvier (Le Genest-St-Isles, France) and housed under standard conditions of temperature (22 ± 1 °C) and lighting (12/12-h light/dark cycles) for 7 days before experiments began. One group of mice (n = 7) was treated with resveratrol; the other (n = 20) was used to prepare blank plasma samples. The study entitled "Measurement of resveratrol in mouse tissues by UHPLC-Synapt" has been approved by the ethical committee of the University Paris Descartes and registered on the number: CEEA34.CHC.043.11.

Resveratrol (15 mg mL^{-1}) was solubilized in an ethanol–water solution (50/50, V V^{-1}). Mice were given vehicle or 150 mg kg^{-1} of resveratrol by gavage. Blood samples were collected into heparinised tubes at 30 and 60 min after administration. The blood samples were centrifuged at 2000 × g for 10 min at 4 °C, and the plasma samples were stored at −80 °C in the dark.