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A simple and sensitive HPLC-UV method for the quantification of piceatannol analog *trans*-3,5,3′,4′-tetramethoxystilbene in rat plasma and its application for a pre-clinical pharmacokinetic study

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ABSTRACT

A simple and sensitive HPLC-UV method was developed and validated for the quantification of piceatannol analog *trans*-3,5,3′,4′-tetramethoxystilbene (M-PIC) in rat plasma. Following protein precipitation with three volumes of acetonitrile, the analytes were separated on a RP-HPLC column, which was protected by a guard column through gradient delivery of a mixture of acetonitrile–water at 40 °C. The UV absorbance at 325 nm was recorded to quantify M-PIC. The retention time of M-PIC and *trans*-3,5-dimethoxystilbene (internal standard) was 7.4 and 8.4 min, respectively. The calibration curves were linear ($R^2 > 0.9989$) with a lower limit of quantification of 15 ng/ml. The intra- and inter-day precisions, in terms of RSD, were all lower than 7.5%. The average analytical recovery ranged from 97.0 to 104.3% while the average absolute recovery ranged from 101.8 to 105.0%. This reliable HPLC method was subsequently applied to assess the pharmacokinetic profile of M-PIC in Sprague–Dawley rats using 2-hydroxypropyl-β-cyclodextrin as a dosing vehicle. The terminal elimination half-life ($t_{1/2\lambda z}$) and clearance (Cl) of M-PIC were 313 ± 20 min and 33.1 ± 3.9 ml/min/kg, respectively; and its absolute oral bioavailability was as high as 50.7 ± 15.0%. M-PIC appeared to have a favorable pharmacokinetic profile and further pharmacological investigation on this phyto-stilbene was warranted.

1. Introduction

Resveratrol (RES, *trans*-3,5,4′-trihydroxystilbene, **1** in Fig. 1), a phytoalexin present in grapes, mulberries and peanuts, has attracted great interests in the past 15 years [1]. Its biological activities, including anti-aging, anti-diabetes, anti-inflammation, anti-obesity, anti-oxidation, cancer chemoprevention, cardio- and neuro-protection have been reported extensively [1,2]. Piceatannol (PIC, *trans*-3,5,3′,4′-tetrahydroxystilbene, **2** in Fig. 1), a well known RES analog, is also present in our diets [3]. Similar to RES, PIC also displayed anti-cancer, anti-inflammatory, anti-oxidant and cardio-protective activities [3–5]. Furthermore, the potency of PIC usually surpasses RES [3,4,6–8]. As RES is converted to PIC by some phase I enzymes in human body [9,10], there lies the possibility for RES to work as a pro-drug for PIC [3].

The pharmacokinetic and metabolic profile of any new drug candidate is one of the key determinants for its success in drug development. In this regard, both RES and PIC lack metabolic stability as they are subjected to extensive phase II metabolism (glucuronide/sulfate conjugation), leading to limited oral bioavailability [1,11,12]. Methylation on the hydroxyl groups of the aromatic ring can avoid the phase II metabolism. Recently, the pharmacokinetic profiles of *trans*-3,5,4′-trimethoxystilbene (fully methylated RES) and pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxystilbene, partially methylated RES) was found to be more favorable than their precursor, RES [13,14]. A fully methylated analog of PIC, *trans*-3,5,3′,4′-tetramethoxystilbene (M-PIC, **3** in Fig. 1), also known as a phytochemical [15], has been recently reported as an anti-allergic, anti-cancer, and anti-inflammatory agent [16–19]. Interestingly, M-PIC also possesses potent inhibitory effects on P-glycoprotein and cyclooxygenases [19,20]. Therefore, M-PIC appears to be an interesting compound for further investigation.

In this study, a simple and sensitive HPLC-UV method was developed and validated for the quantification of M-PIC in rat plasma. This reliable method was subsequently applied to assess the pharmacokinetic profile of M-PIC in Sprague–Dawley rats after single oral or intravenous administration. To our knowledge, this is the first report on the pharmacokinetics of M-PIC. Our study indicated that M-PIC had a superior pharmacokinetic profile over its precursor, PIC.

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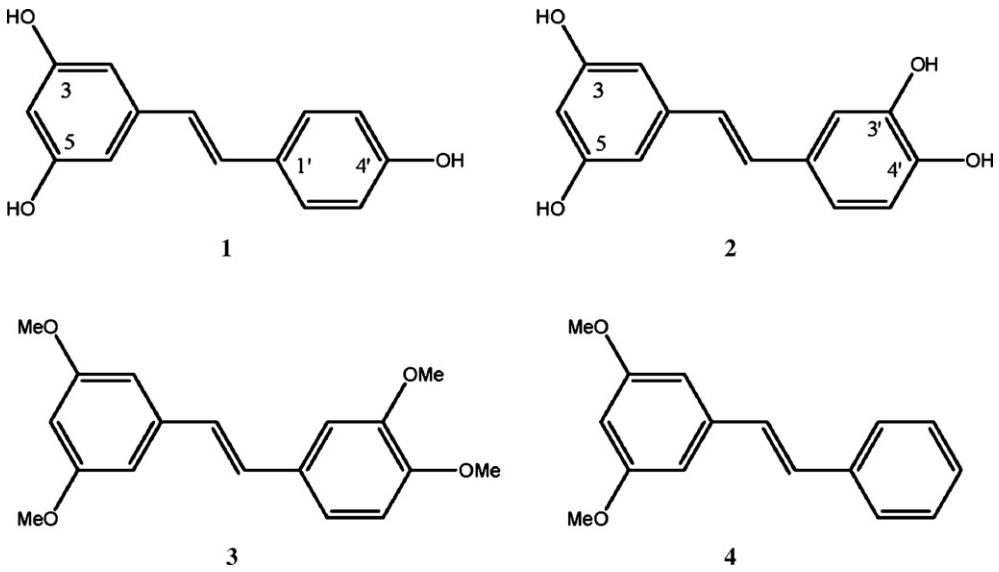

Fig. 1. Chemical structures of resveratrol (**1**), piceatannol (**2**), *trans*-3,5,3′,4′-tetramethoxystilbene (**3**) and *trans*-3,5-dimethoxystilbene (**4**, internal standard).

2. Experimental

2.1. Special precaution

As stilbenes are light sensitive, all laboratory procedures involving the manipulations of M-PIC and *trans*-3,5-dimethoxystilbene were executed in a dimly lit environment.

2.2. Chemicals and reagents

Trans-3,5,3′,4′-tetramethoxy-stilbene (M-PIC, purity > 98%, determined by HPLC) was prepared by some of us employing a previously reported method [21]. *Trans*-3,5-dimethoxystilbene (DMS, **4** in Fig. 1, internal standard, purity > 98%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CyD) (degree of substitution: ∼0.6) was a generous gift from Roquette Freres S.A. (Lestrem, France). Gradient grade acetonitrile (Merck, Darmstadt, Germany) and Milli-Q water (18.2 MΩ cm at 25 °C) was used to prepare mobile phase. Other solvents or reagents were at least of analytical grade.

2.3. Chromatographic conditions

HPLC analyses were carried out on a Shimadzu (Kyoto, Japan) 2010A liquid chromatography system, which comprised of a quaternary gradient low-pressure mixing pump, an online degasser, an auto-sampler, a column oven, a dual-wavelength UV–vis detector and a system controller. The software Class-VP Version 6.12 SP1 (Shimadzu, Kyoto, Japan) was used for system control and data processing.

A RP-HPLC column (Agilent ZORBAX Eclipse Plus C18: 250 mm × 4.6 mm i.d., 5 μm), which was protected by a guard column (Agilent ZORBAX Eclipse Plus C18: 12.5 mm × 4.6 mm i.d., 5 μm) was selected to quantify M-PIC in plasma. Chromatographic separation was obtained through a 15-min gradient delivery of a mixture of acetonitrile and Milli-Q water at a flow rate of 1.2 ml/ml at 40 °C. The gradient schedule was: (a) 0–1.5 min, acetonitrile: 55%; (b) 1.5–5.5 min, acetonitrile: 55 → 90%; (c) 5.5–10 min, acetonitrile: 90%; (d) 10–15 min, acetonitrile: 55%. UV absorbance at both 325 and 300 nm was recorded but only the data acquired at 325 nm was applied to set up the assay.

2.4. Sample preparation

Stock solution of M-PIC was prepared in DMSO to obtain a final concentration of 1 mg/ml weekly. This stock solution was stored at room temperature (24 °C) and protected from light. The calibration standards for rat plasma were prepared through serial dilution of the M-PIC stock with pooled blank rat plasma. The internal standard (DMS) was dissolved in acetonitrile and diluted to 150 ng/ml (working solution). During sample preparation, three volumes of DMS–acetonitrile working solution were added to one volume of rat plasma. After vigorous vortexing for 20 s, the samples were centrifuged at 10,000 × *g* for 10 min at 4 °C. Finally, the supernatant was transferred into a glass insert that was pre-installed in a 1.5 ml auto-sampler vial. This simple protocol for protein precipitation had been used in the HPLC analyses for *trans*-3,5,4′-trimethoxystilbene and pterostilbene [13,14]. During each assay, 50 μl supernatant was injected into the HPLC system. The minimal volume of plasma required for an analysis was 30 μl.

2.5. Method validation

This HPLC method was validated with regards its selectivity, linearity, precision (intra- and inter-day), sensitivity, accuracy, absolute recovery and stability.

The selectivity was initially investigated by comparing the chromatogram from the pooled blank rat plasma and the chromatogram from the same sample but spiked with M-PIC and DMS. In the subsequent pharmacokinetic study, pre-dosing plasma sample was also collected from each individual rat ($n = 7$). The selectivity of this assay was further documented through a chromatographic comparison between the pre-dosing and post-dosing plasma samples.

The ratio between the peak area of M-PIC and that of DMS (internal standard) was defined as the analytical response. Linear regression was carried out with GraphPad Prism Version 5.01 (La Jolla, CA 92037 USA) via least sum-of-squares method, where x was the concentration of M-PIC, y was the analytical response, and $1/x^2$ was used as a weighting factor [13,14]. The calibration standards of the following concentrations 15, 50, 100, 250, 500, 1000, 1500, 2000 and 2500 ng/ml were used to assess linearity. The plasma M-PIC levels in the subsequent pharmacokinetic study were all covered by this calibration range.