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Short communication

# Determination of *trans*-2,4,3′,4′,5′-pentamethoxystilbene in rat plasma and its application to a pharmacokinetic study

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ABSTRACT

*trans*-2,4,3′,4′,5′-Pentamethoxystilbene (2,4,3′,4′,5′-PMS) is a resveratrol derivative that displayed promising pre-clinical anti-cancer activities. In this study, a simple HPLC method was developed and validated to determine 2,4,3′,4′,5′-PMS in rat plasma. The lower limit of quantification was 9 ng/ml. The intra- and inter-day precision in terms of relative standard deviation was less than 9.7% and the bias rate ranged from −6.4 to +7.8%. The pharmacokinetics of 2,4,3′,4′,5′-PMS was subsequently studied in Sprague-Dawley rats. Upon intravenous administration (0.75 mg/kg), 2,4,3′,4′,5′-PMS displayed moderate clearance (58.5 ± 19.5 ml/min/kg) and terminal elimination half-life (147 ± 61 min). Aqueous solubility appeared to be a barrier to oral absorption. When suspension was given (4 mg/kg), the absolute oral bioavailability was almost nil; when 2,4,3′,4′,5′-PMS was fully solubilized by randomly methylated-β-cyclodextrin, it possessed a low bioavailability (3.63 ± 2.06%). The pharmacokinetic comparison among 2,4,3′,4′,5′-PMS and other methoxylated stilbenes suggested that the 2-methoxy group was unfavorable to oral bioavailability. Future investigations on 2,4,3′,4′,5′-PMS should be focused on chemo-prevention of colorectal carcinogenesis.



## 1. Introduction

Resveratrol (*trans*-3,5,4′-trihydroxystilbene, **1**, Fig. 1) is a dietary phytoalexin that possesses pleiotropic health-promoting activities such as anti-ageing, anti-diabetic, anti-inflammation, anti-obesity, anti-oxidation, cancer chemoprevention, cardio- and neuro-protection [1]. *trans*-2,4,3′,4′,5′-Pentamethoxystilbene (2,4,3′,4′,5′-PMS, **2**, Fig. 1) is a synthetic resveratrol analogue with potent anti-cancer activities. It inhibited human cytochrome P450 (CYP) 1A1 and 1B1, two enzymes involved in carcinogenesis with potencies at least 10-fold stronger than that of resveratrol [2–4]. In colon cancer cell lines, it also displayed a superior anti-proliferative effect than resveratrol [5]. The apoptotic cell death was induced by 2,4,3′,4′,5′-PMS through enhancing the polymerization of microtubules [5]. The *in vivo* anti-cancer efficacy of 2,4,3′,4′,5′-PMS has also been confirmed in mouse xenograft model [5]. Moreover, in a preventive study, it suppressed colitis-associated colon carcinogenesis in mice [6]. Clearly, 2,4,3′,4′,5′-PMS has appeared as a promising chemotherapeutic/chemo-preventive candidate for colon cancer.

The pharmacokinetic profiles of several methylated resveratrol analogues were reported recently [7–15]. Generally, HPLC–UV methods could measure these compounds with acceptable sensitivity [9–15]. A simple and sensitive HPLC–UV method was therefore developed and validated for the determination of 2,4,3′,4′,5′-PMS in rat plasma in the present study. The pharmacokinetic profiles of 2,4,3′,4′,5′-PMS was assessed after single intravenous and oral administration in Sprague-Dawley rats. The pharmacokinetic profiles of 2,4,3′,4′,5′-PMS were subsequently compared to those of its isomer, *trans*-3,5,3′,4′,5′-pentamethoxystilbene (3,5,3′,4′,5′-PMS, also known as MR-5, **3**, Fig. 1) as well as other stilbenes. To the authors' knowledge, this is the first report on the pharmacokinetics of 2,4,3′,4′,5′-PMS. Findings from this study would be useful to elucidate the structure–pharmacokinetic relationship of stilbenes and to evaluate the potential medicinal application of 2,4,3′,4′,5′-PMS.

## 2. Meterials and methods

### 2.1. Special precautions

All laboratory procedures involving the manipulation of 2,4,3′,4′,5′-PMS and *trans*-stilbene were executed in a dimly lit environment.

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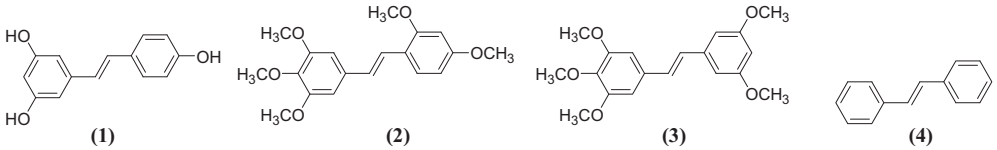


**Fig. 1.** Chemical structures of resveratrol (**1**), *trans*-2,4,3′,4′,5′-pentamethoxystilbene (**2**), *trans*-3,5,3′,4′,5′-pentamethoxystilbene (**3**), and *trans*-stilbene (**4**).

### 2.2. Chemicals and reagents

*trans*-2,4,3′,4′,5′-Pentamethoxystilbene (2,4,3′,4′,5′-PMS, **2**, Fig. 1) was synthesized employing a previously published method based on an Arbuzov rearrangement followed by the Horner–Emmons–Wadsworth reactions [5,6]. Purity was established higher than 97% by HPLC. To the best of our knowledge, the NMR spectral properties of **2** have not been previously reported and are as follows: $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.49 (d, 8.5 Hz, 1H), 7.28 (d, 16.0 Hz, 1H, partially overlapped with $CHCl_3$ signal), 6.95 (d, 16.0 Hz, 1H), 6.74 (s, 2H), 6.54 (dd, 8.5 Hz, 2.0 Hz, 1H), 6.49 (d, 2.0 Hz, 1H), 3.92 (s, 6H), 3.89 (s, 3H), 3.87 (s, 3H), 3.85 (s, 3H). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 160.5, 158.1, 153.3, 137.5, 134.13, 127.3, 127.1, 122.9, 119.4, 105.0, 103.4, 98.5, 60.9, 56.2, 55.5, 55.4. *trans*-Stilbene (internal standard, purity: 96%, **4**, Fig. 1) and sodium salt of carboxymethylcellulose (CMC) were obtained from Sigma–Aldrich (St. Louis, MO 63178, USA). 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD, degree of substitution: about 0.6) and randomly methylated-β-cyclodextrin (RM-β-CD, degree of substitution: about 1.8) were kindly donated from Roquette (Lestrem, France) and Wacker (Burghausen, Germany), respectively.

### 2.3. Liquid chromatography

The chromatographic system used in the development and validation of this assay was a Shimadzu (Kyoto, Japan) 2010A Liquid Chromatography. It consists 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 HPLC was controlled by a personal computer through the software of Shimadzu Class-VP Version 6.12 SP1 (Shimadzu, Kyoto, Japan). Peak integration was also executed by the same software.

The chromatographic conditions were modified from our recent methods for the quantification of *trans*-3,5,3′,4′,5′-pentamethoxystilbene (3,5,3′,4′,5′-PMS) and *trans*-3,5,4,4′-tetramethoxystilbene [11,15]. The chromatographic separation was achieved on a reversed phase 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) through a 12-min gradient delivery of a mixture of acetonitrile and water at a flow rate of 1.5 ml/min at 50 °C. The gradient schedule was: (a) 0–3.5 min, acetonitrile: 57.5%; (b) 3.5–5 min, acetonitrile: 57.5 → 90%; (c) 5–9 min, acetonitrile: 90%; (d) 9–12 min, acetonitrile: 57.5%. Ultraviolet (UV) absorbance at 329 (maximal UV absorption wavelength) and 300 nm was recorded while only the data acquired at 329 nm was used for the quantification of 2,4,3′,4′,5′-PMS.

### 2.4. Sample preparation

2,4,3′,4′,5′-PMS was dissolved in DMSO and diluted to 1.00 mg/ml. This stock solution was dispensed into individual vials and stored at room temperature (25 °C). The calibration standards (9, 50, 100, 250, 500, 1000 and 1500 ng/ml) or quality control (QC) samples (40, 400 and 1400 ng/ml) were prepared by diluting the stock solution with blank rat plasma. The *trans*-stilbene (internal standard) was dissolved in acetonitrile and diluted to 300 ng/ml (working solution). During sample preparation, three volumes of *trans*-stilbene–acetonitrile working solution were mixed with one volume of rat plasma. After vortex, the samples were centrifuged at 10,000 × *g* for 5 min at 4 °C. After centrifugation, the supernatant was carefully transferred into a glass insert pre-installed in a 1.5 ml auto-sampler vial. During HPLC assay, 75 μl analyte was injected into the system. Only 40 μl plasma was required for a single assay.

### 2.5. Assay validation

This HPLC method was validated by assessing its specificity, sensitivity, linearity ($R^2$), precision (intra- and inter-day), accuracy (bias rate), absolute recovery and the stability profiles of 2,4,3′,4′,5′-PMS. The specificity was examined by comparing the chromatograms of 6 individual blank rat plasma samples and the corresponding plasma samples spiked with 2,4,3′,4′,5′-PMS and *trans*-stilbene. The specificity of the assay was further contested with the actual pharmacokinetic samples by chromatographic comparison between pre-dosing and post-dosing plasma samples ($n$ = 14). The assay sensitivity was represented by lower limit of detection (LOD) and lower limit of quantification (LOQ), which were defined as a signal to noise ratio equal to 3 and 10, respectively. The ratio between the peak area of 2,4,3′,4′,5′-PMS and *trans*-stilbene (internal standard) (λ = 329 nm) was defined as the analytical response. Linear regression was carried out with GraphPad Prism Version 5.04 (La Jolla, CA 92037, USA), where $x$ was the concentration of 2,4,3′,4′,5′-PMS, $y$ was the analytical response, and $1/x^2$ was used as a weighting factor [11,15]. The calibration standards of the following concentrations 9, 50, 100, 250, 500, 1000 and 1500 ng/ml were used to assess the linearity. The calibration was executed in 5 consecutive days. For intra-day analysis, 5 replicates of samples were analyzed; for inter-day assay, duplicate samples were analyzed. The intra- and inter-day relative standard deviation (RSD) of analytical response at individual concentration was calculated and applied to indicate assay precision. The QC samples (40, 400 and 1400 ng/ml) were prepared and analyzed in the same way. The precision (RSD), absolute recovery (%) and accuracy (bias rate) were measured with the QC samples. The absolute recovery of 2,4,3′,4′,5′-PMS was calculated by comparing the peak areas of 2,4,3′,4′,5′-PMS in the spiked plasma samples with plasma-free samples containing the same amount of 2,4,3′,4′,5′-PMS [10,12]. The absolute recovery of *trans*-stilbene (internal standard) was measured in the same way. The bias (%) was calculated as [9]:

$$\text{Bias}\,(\%) = \left(1 - \frac{\text{2, 4, 3′, 4′, 5′-PMS}_{\text{Measured}}}{\text{2, 4, 3′, 4′, 5′-PMS}_{\text{Spiked}}}\right) \times 100\%$$

The stability of 2,4,3′,4′,5′-PMS in DMSO solution was evaluated after storage at room temperature (25 °C) for 12 days. The stability of 2,4,3′,4′,5′-PMS in rat plasma under different storage conditions was also assessed with the QC samples. The impact of freeze–thaw on stability was assessed after three freeze (−80 °C)–thaw (25 °C) cycles. Short-term refrigerator storage stability (4 °C, 24 h) and long-term deep freezer storage stability (−80 °C, 12 days) was also examined. The post-preparative stability study at room temperature in auto-sampler vial was investigated by reanalyzing the samples one day later.

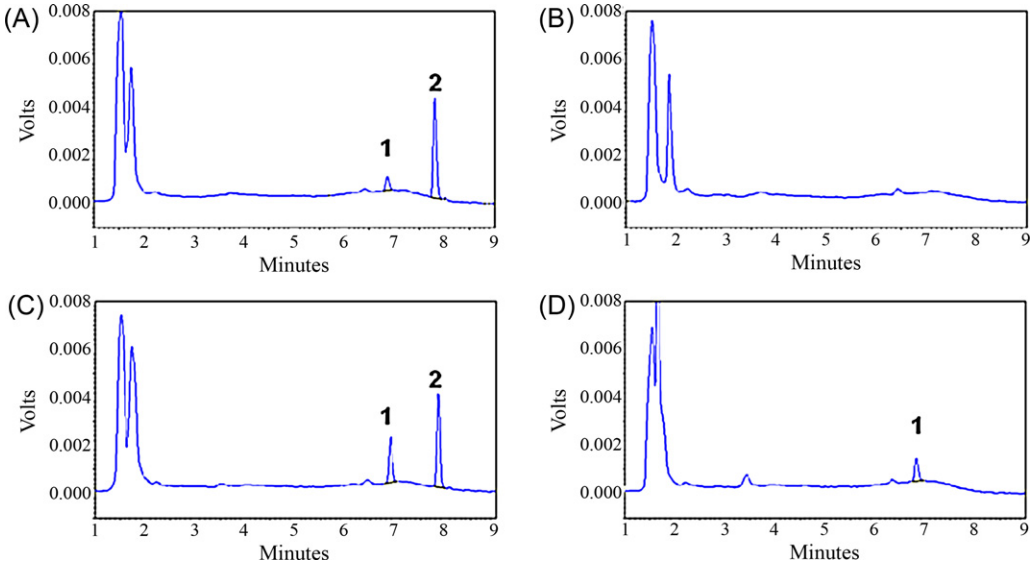


**Fig. 2.** Typical chromatograms (UV absorbance, λ = 329 nm) of (**A**) a blank plasma sample spiked with 2,4,3′,4′,5′-PMS (40 ng/ml) and *trans*-stilbene (internal standard) (900 ng/ml), (**B**) a pre-dosing plasma sample, (**C**) a plasma sample taken from a rat at 15 min after receiving an intravenous dose of 2,4,3′,4′,5′-PMS (0.75 mg/kg) (with internal standard), and (**D**) a plasma sample taken from a rat at 30 min after receiving an oral dose of 2,4,3′,4′,5′-PMS (4 mg/kg) (without internal standard). Peak 1: 2,4,3′,4′,5′-PMS; peak 2: internal standard.

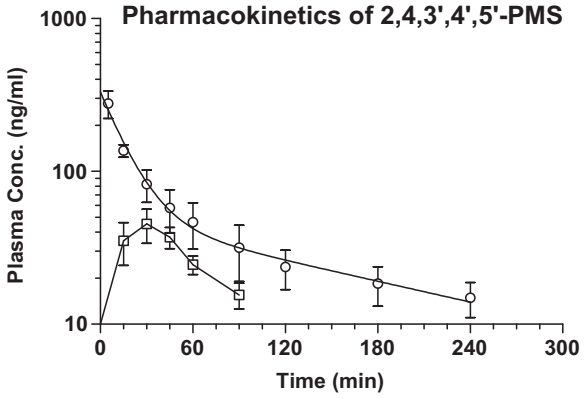


**Fig. 3.** Plasma pharmacokinetic profiles of 2,4,3′,4′,5′-PMS in Sprague-Dawley rats. Symbols represent mean values and error bars represent SD. Intravenous administration (open circles): 0.75 mg/kg, $n = 4$, except $n = 3$: at 240 min; oral administration (open squares): 4 mg/kg, $n = 5$: at 15 and 30 min, $n = 4$: at 45 and 60 min, $n = 3$: at 90 min.

### 2.6. Pharmacokinetic study

The animal handling protocol had been reviewed and approved by the Institutional Animal Care and Use Committee of the National University of Singapore (NUS). This animal model has been used extensively to assess the pharmacokinetics of various stilbenes [9–17]. Fourteen Sprague-Dawley rats (male, 7–8 weeks old, 300–320 g, bred by the Center for Animal Resources, NUS) were divided into three groups. Group 1 ($n = 4$) received a single bolus intravenous administration of 2,4,3′,4′,5′-PMS (0.75 mg/kg), serial blood samples (150 μl per sample) were collected before dosing and at 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min post dosing. Group 2 ($n = 5$) received a single oral administration of 2,4,3′,4′,5′-PMS in CMC suspension (4 mg/kg) through gavage and serial blood samples were collected before dosing and at 30, 60, 90, 120, 150, 180, 240, 300, 420 and 540 min post administration. Group 3 ($n = 5$) received a single oral administration of 2,4,3′,4′,5′-PMS in RM-β-CD solution (4 mg/kg) through gavage and serial blood samples were collected before dosing and at 15, 30, 45, 60, 90, 120, 180, 300, 420 and 540 min post dosing. The harvested plasma was kept at −80 °C until HPLC assay, which was performed within 5 days after the *in vivo* study.

Pharmacokinetic analysis was performed by WinNonlin standard version 1.0 (Scientific Consulting Inc., Apex, NC 27502, USA). As the intravenous plasma pharmacokinetic profile of 2,4,3′,4′,5′-PMS displayed a typical bi-exponential decline, the plasma 2,4,3′,4′,5′-PMS concentration–time data was fitted into the classical two-compartment first-order open model ($C = Ae^{-\alpha t} + Be^{-\beta t}$) using nonlinear least squares method with a weighting factor of $1/y^2$ as described previously [17]. The plasma exposure (area under the plasma concentration–time curve ($AUC_{0\rightarrow last}$)), clearance ($Cl_{0\rightarrow last}$), terminal elimination half-life ($t_{1/2\lambda Z}$) and mean transition time ($MTT_{0\rightarrow last}$) was calculated through non-compartmental method [17].

## 3. Results and discussion

### 3.1. Assay validation

The specificity of this simple HPLC method for the determination of 2,4,3′,4′,5′-PMS in rat plasma was demonstrated. Under our separation conditions, 2,4,3′,4′,5′-PMS and *trans*-stilbene eluted at about 6.8 and 7.8 min, respectively (Fig. 2**A**). We did not observe any notable interference peak in the chromatograms acquired from either blank plasma samples ($n = 6$) or pre-dosing plasma samples ($n = 14$) (a typical chromatogram of a pre-dosing sample is shown in Fig. 2**B**). Furthermore, no notable metabolite or interference peak was identified in the chromatograms acquired from the rats received 2,4,3′,4′,5′-PMS dosing (Fig. 2**C** and **D**).

The lower LOD and LOQ of 2,4,3′,4′,5′-PMS, indicators of the sensitivity of the assay were found to be 3 and 9 ng/ml, respectively. The calibration curves were all linear with regression correlation coefficients ($R^2$) > 0.9969. The intra-day calibration equation was: $y = 0.004436x - 0.005090$ while the inter-day calibration was: $y = (0.004605 \pm 0.000123)x - (0.007070 \pm 0.003972)$. The precision of the HPLC assay was confirmed and the intra-day or inter-day RSDs that were all less than 9.7% (calibration data is not shown, QC data is shown in Table 1). The accuracy of the assay was also demonstrated as the bias rates ranged from −6.4 ± 7.8% at all concentrations in the QC samples (Table 1). The absolute recovery rates

**Table 1**
Analytical accuracy and precision of 2,4,3′,4′,5′-PMS in rat plasma.[a]

| Amount spiked (ng/ml) | Intra-day | | | Inter-day | | |
|---|---|---|---|---|---|---|
| | Amount measured (ng/ml) | Precision (RSD, %) | Bias range (%) | Amount measured (ng/ml) | Precision (RSD, %) | Bias range (%) |
| 40.0 | 39.1 ± 1.1 | 2.7 | −4.4 to +2.4 | 39.8 ± 1.4 | 3.5 | −4.4 to +4.1 |
| 400.0 | 387.2 ± 7.6 | 2.0 | −4.8 to 0.0 | 387.2 ± 5.6 | 1.5 | −5.1 to − 0.0 |
| 1400.0 | 1333.2 ± 14.3 | 1.1 | −6.0 to 3.3 | 1390.3 ± 77.3 | 5.6 | −6.4 to +7.8 |

[a] $n = 5$.

**Table 2**
Stability profiles of 2,4,3′,4′,5′-PMS.[a]

| Stability (% remained) | Spiked concentration (ng/ml) | | |
|---|---|---|---|
| | 40 | 400 | 1400 |
| Stock solution stored at 24 °C for 12 days | 99.7 ± 2.0 | 98.9 ± 1.0 | 100.6 ± 0.6 |
| Plasma samples stored at 4 °C for 24 h | 95.3 ± 1.6 | 95.5 ± 1.0 | 96.2 ± 1.0 |
| Post-preparative samples stored at 24 °C for 24 h | 95.7 ± 3.7 | 98.0 ± 1.7 | 96.6 ± 1.3 |
| Plasma samples after three freeze–thaw cycles | 95.5 ± 3.3 | 96.0 ± 3.4 | 95.0 ± 1.5 |
| Plasma samples stored at −80 °C for 12 days | 95.6 ± 5.7 | 96.2 ± 1.8 | 95.2 ± 1.0 |

[a] Results were presented as mean ± SD ($n = 5$).

at the concentrations of 40, 400 and 1400 ng/ml were 99.9 ± 2.8%, 105.1 ± 2.0% and 102.9 ± 1.1%, respectively ($n = 5$). The absolute recovery rate of internal standard (*trans*-stilbene) was 103.6 ± 1.5% ($n = 15$). The stability profiles of 2,4,3′,4′,5′-PMS were also assessed and 2,4,3′,4′,5′-PMS appeared to be stable under the tested conditions (Table 2).

In summary, a simple HPLC method was developed and validated for the quantification of 2,4,3′,4′,5′-PMS in rat plasma. This method was established by re-optimizing the chromatographic conditions applied in the quantification of *trans*-3,5,3′,4′,5′-pentamethoxystilbene, *trans*-3,5,4,4′-tetramethoxystilbene as well as several other methoxylated stilbenes [9–15]. In comparison to the previous methods, the assay sensitivity of the current method was slightly improved and the lower LOQ was decreased from 15 ng/ml to 9 ng/ml. This HPLC method was subsequently applied in the pharmacokinetic study of 2,4,3′,4′,5′-PMS.

**Table 3**
Pharmacokinetic parameters of 2,4,3′,4′,5′-PMS.[a]

| Parameters | Intravenous ($n = 4$) | Oral ($n = 5$) |
|---|---|---|
| Formulation | HP-β-CD | RM-β-CD |
| Dose (mg/kg) | 0.75 | 4 |
| $A$ (ng/ml) | 299 ± 111 | – |
| $B$ (ng/ml) | 32.9 ± 11.8 | – |
| $\alpha$ ($\times 10^{-2}$ min$^{-1}$) | 6.15 ± 3.35 | – |
| $\beta$ ($\times 10^{-3}$ min$^{-1}$) | 4.40 ± 2.28 | – |
| $V_c$ (l/kg) | 2.49 ± 1.00 | – |
| $AUC_{0\rightarrow last}$ ($\times 10^4$ min ng/ml) | 1.16 ± 0.46 | 0.224 ± 0.127 |
| Cl (ml/min/kg) | 58.5 ± 19.5 | – |
| $t_{1/2\,\lambda Z}$ (min) | 147 ± 61 | – |
| $MTT_{0\rightarrow last}$ (min) | 60.9 ± 26.1 | – |
| $C_{max}$ (ng/ml) | – | 53.2 ± 15.1 |
| $t_{max}$ (min) | – | 15 or 30 |
| $F$ (%) | | 3.63 ± 2.06 |

[a] Results were presented as mean ± SD.

### 3.2. Application to a pharmacokinetic study

As methoxylated stilbenes possesses poor aqueous solubility, cyclodextrins such as HP-β-CD and RM-β-CD were applied to form water-soluble formulations of 2,4,3′,4′,5′-PMS. The intravenous pharmacokinetic profile was assessed after bolus injection of 2,4,3′,4′,5′-PMS (0.75 mg/kg) solubilized with 0.3 M HP-β-CD. The plasma 2,4,3′,4′,5′-PMS *versus* time profile is shown in Fig. 3. Upon intravenous injection, plasma 2,4,3′,4′,5′-PMS levels declined through a bi-exponential process, i.e. a distribution phase followed by a terminal elimination phase. Therefore, the classical two-compartment first-order open model was selected to describe the intravenous pharmacokinetic profile of 2,4,3′,4′,5′-PMS. The plasma 2,4,3′,4′,5′-PMS *versus* time data of individual rat was fitted into the model and the fitting was good ($R^2 = 0.9785$, 0.9998, 0.9957 and 0.9964, respectively), indicating that an appropriate model was chosen. The pharmacokinetic parameters of 2,4,3′,4′,5′-PMS are listed in Table 3. 2,4,3′,4′,5′-PMS possessed a moderate apparent volume of distribution of the central compartment ($V_c = 2.49 \pm 1.00$ l/kg), clearance (Cl = 58.5 ± 19.5 ml/min/kg), terminal elimination half-life ($t_{1/2\lambda z} = 147 \pm 61$ min) and mean transition time (MTT = 60.9 ± 26.1 min). The plasma 2,4,3′,4′,5′-PMS concentration dropped to unquantifiable levels (<9 ng/ml) 4 or 5 h after intravenous administration. However, 2,4,3′,4′,5′-PMS was still detectable (>3 ng/ml) at 6 h after dosing. Clearly, the elimination of 2,4,3′,4′,5′-PMS was moderately slow.

As the aqueous solubility of resveratrol and *trans*-3,5,4′-trimethoxystilbene (resveratrol trimethyl ether) had significant influence on their oral pharmacokinetics [16,17], the impact of aqueous solubility on the oral bioavailability of 2,4,3′,4′,5′-PMS was also assessed with two formulations, namely 2,4,3′,4′,5′-PMS suspended in 0.3% CMC and 2,4,3′,4′,5′-PMS solubilized with 0.3 M RM-β-CD. When suspension was given, 2,4,3′,4′,5′-PMS was not quantifiable in almost all post-dosing plasma samples (<9 ng/ml). It was only detected in a sample collected at 2 h post-gavage (in-between 3–9 ng/ml). Clearly, the absolute oral bioavailability of 2,4,3′,4′,5′-PMS was almost nil when it was given in an insoluble form. The oral pharmacokinetic profile of 2,4,3′,4′,5′-PMS solubilized in 0.3 RM-β-CD is shown in Fig. 3. When given in a solution form, 2,4,3′,4′,5′-PMS was absorbed rapidly after oral dosing and the plasma concentration peaked within 15–30 min. However, the maximal plasma concentration ($C_{max}$) was fairly low ($C_{max} = 53.2 \pm 15.1$ ng/ml). After achieving $C_{max}$, the 2,4,3′,4′,5′-PMS plasma concentrations dropped to unquantifiable within 1–2 h after oral gavage. The absolute oral bioavailability was poor ($F = 3.63 \pm 2.06\%$). Clearly, aqueous solubility appeared to be one of the barriers to the oral absorption of 2,4,3′,4′,5′-PMS and solubility enhancive excipient(s) may allow oral absorption of 2,4,3′,4′,5′-PMS in a small extent for its potential clinical effects.

The pre-clinical pharmacokinetic profiles of resveratrol, pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxystilbene) and several complete methoxylated stilbenes have been assessed with similar approaches recently [9–17]. A pharmacokinetic

comparison among these compounds may offer insight into the structure-pharmacokinetic relationship of stilbenes. The intravenous pharmacokinetic profile of 2,4,3′,4′,5′-PMS was more favorable than that of resveratrol [16], i.e. 2,4,3′,4′,5′-PMS had more plasma exposure, longer $t_{1/2\lambda z}$, longer MTT and slower Cl. Also, it is of note that the values of the major intravenous pharmacokinetic parameters of 2,4,3′,4′,5′-PMS, namely $V_c$, $t_{1/2\lambda z}$, MTT and Cl were comparable to those values of the other complete methoxylated stilbenes including *trans*-3,5,4′-trimethoxystilbene [10,17], *cis*-3,5,4′-trimethoxystilbene [14], *trans*-3,4,3′,5′-tetramethoxystilbene [12], *trans*-2,4,3′,5′-tetramethoxystilbene [9], *trans*-3,5,4,4′-tetramethoxystilbene [15], and 3,5,3′,4′,5′-PMS [11]. Based on these studies, it is concluded that methylation of the hydroxyl groups of the stilbene offers better metabolic stability and subsequently leads to more favorable intravenous pharmacokinetic profile.

The absolute oral bioavailability of 2,4,3′,4′,5′-PMS was low. Even given in a fully soluble form, its *F* was only 3.63 ± 2.06%. It is generally believed that aqueous solubility, membrane permeability and metabolic stability are the key determinants of oral bioavailability of a given compound [18,19]. When RM-β-CD was applied to deliver 2,4,3′,4′,5′-PMS, the solubility barrier no longer existed. The pharmacokinetic profiles of 3,5,3′,4′,5′-PMS, an isomer of 2,4,3′,4′,5′-PMS have been studied by the authors recently [11]. Interestingly, the oral pharmacokinetics of 3,5,3′,4′,5′-PMS was quite favorable when water-soluble formulation was applied. A side by side pharmacokinetic comparison between these two pentamethoxystilbenes may reveal the mechanism that leads to the difference in oral bioavailability. The major intravenous pharmacokinetic parameters including $V_c$, $t_{1/2\lambda z}$, MTT and Cl were similar in both compounds ($P > 0.05$, two-tailed independent *t*-test), indicating these two stilbenes possessed similar distribution, metabolic and/or elimination profiles. However, the $C_{max}$, oral plasma exposure and *F* of 3,5,3′,4′,5′-PMS was about 2-, 9- and 7-fold higher than that of 2,4,3′,4′,5′-PMS, respectively ($P < 0.01$, two-tailed independent *t*-test). As cyclodextrin formulations were applied, the solubility issue was ruled out. Since the metabolic and/or elimination profiles of these two pentamethoxystilbenes appeared to be similar, the difference in oral bioavailability should be attributed to the membrane permeability problem. When water-soluble cyclodextrin formulations were given, both *trans*-3,5,4′-trimethoxystilbene and *trans*-3,4,3′,5′-tetramethoxystilbene displayed good bioavailability as high as about 50% [10,12,17], indicating that the 4-methoxy group was unlikely to cause permeability problem. Therefore, the 2-methoxy group of the stilbene appeared to be unfavorable to the membrane permeability. Similar result has been observed with *trans*-2,4,3′,5′-tetramethoxystilbene [9]. Although its metabolism and/or elimination was not fast, the *F* of *trans*-2,4,3′,5′-tetramethoxystilbene was as low as 4.5 ± 3.2% [9]. In future design of new resveratrol analogues, 2-methoxyl group or other modification on the 2-position of stilbene are not recommended as such structure may cause membrane permeability problem.

The total number of methoxy groups might also have some impact on the membrane permeability. Using *trans*-3,5,4′-trimethoxystilbene, *trans*-3,4,3′,5′-tetramethoxystilbene and 3,5,3′,4′,5′-PMS as an example, when the total number of methoxy groups increases from 3 or 4 to 5, the *F* dropped from about 50% to about 30% [10–12,17]. However, the impact of the total number of methoxy groups may not be as dominant as its location.

According to Amidon's Biopharmaceutics Classification System [20], 2,4,3′,4′,5′-PMS and 3,5,3′,4′,5′-PMS could be allocated into the Class II and Class IV substance, respectively. Therefore, solubility-enhancive excipient(s) may be crucial to improve the oral absorption of these two pentamethoxystilbenes. For therapeutic intervention, 3,5,3′,4′,5′-PMS appeared to be more favorable than 2,4,3′,4′,5′-PMS as its oral bioavailability was as high as about 30% when the solubility barrier was overcame [11].

The 50% inhibitory concentrations ($IC_{50}$) of 2,4,3′,4′,5′-PMS on cytochrome p450 1B1 (CYP 1B1) was 21.3 ± 1.5 ng/ml and it could be achieved in plasma within the first hour after oral administration [4]. Such systemic 2,4,3′,4′,5′-PMS exposure might provide some cancer chemo-preventive benefit. Moreover, although 2,4,3′,4′,5′-PMS did not possess much systemic exposure in rats, it inhibited colitis-associated colorectal carcinogenesis in mice when it was given orally using 5% DMSO (v/v) in olive oil as a dosing vehicle [6]. Therefore, abundant systemic exposure may be unnecessary for the prevention of colorectal carcinogenesis and further investigation of 2,4,3′,4′,5′-PMS as chemo-preventive agent in colorectal carcinogenesis is warranted.

## 4. Conclusions

In summary, a simple and sensitive HPLC method has been developed and validated to determine 2,4,3′,4′,5′-PMS in rat plasma. This HPLC method was successfully applied to study the pharmacokinetic profiles of 2,4,3′,4′,5′-PMS in Sprague-Dawley rats. Although 2,4,3′,4′,5′-PMS had appropriate intravenous pharmacokinetic profile, its oral bioavailability was low. Aqueous solubility and the 2-methoxy group appeared to be barriers to its oral bioavailability. Future investigations on 2,4,3′,4′,5′-PMS should be focused on chemo-prevention of colorectal carcinogenesis.

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