SUPPLEMENTARY DATA

Instrumental analysis

Samples were analyzed on a 20A high performance liquid chromatography system (Shimadzu, Japan) coupled with an AB Sciex Q-Trap 4500 mass spectrometer (Applied Biosystems, Foster City, CA, USA). The chromatographic separation was performed on a BETASIL Phenyl Column (2.1mm×150mm, 3um, Thermo Scientific, USA). Analysis was completed with a gradient elution of acetonitrile (A)-0.1% aqueous acetic acid (B) within 10 min at a flow rate of 0.4 mL/min. The gradient was as follows: 0-0.3 min, 5% A; 0.3-1.5 min, 5% A → 60% A; 1.5-5 min, 60% A; 5-5.5 min, 60% A → 5% A; 5.5-10 min, 5% A. The injection volume was 5 μL.

The source temperature was set at 550 °C and the ionization voltage was -4500 V. Both the nebulizer gas (GS1) pressure and the turbo heater gas (GS2) pressure were 55 psi. DHPPA and syringic acid (internal standard, IS) were quantitated by multiple reaction monitoring mode with negative electrospray ionization. The optimized mass transition ion-pairs (m/z) for quantitation were 181.1→95 for DHPPA, and 196.9→121 for syringic acid respectively.

Method Validation

The current method validation was performed according to European Medicines Agency Guideline on Bioanalytical Method Validation with respect to selectivity, lower limit of quantification (LLOQ), calibration curve, accuracy, precision, recovery, matrix effect, and stability.

Selectivity was assessed by comparing chromatograms of blank plasma samples with that of corresponding spiked plasma samples and plasma samples obtained from the participants. As shown in Supplemental Figure 1, the retention times of DHPPA and Syringic acid were 2.60 min and 2.67 min respectively. No significant endogenous interference was observed at the retention times of the analyte and IS.

Calibration curves prepared by assaying standard plasma samples at seven concentrations were constructed from the peak area ratios of the analyte to IS versus plasma concentrations using a $1/x^2$ weighted linear least-squares regression model. The calibration curve exhibited good linearity over the concentration range of 0.4-50 ng/mL for DHPPA. The regression equation obtained was $Y=80.1364X+161.960$ ($r^2=0.9997$) for DHPPA. The lower limit of quantification of DHPPA which was calculated on the basis of a signal-to-noise ratio of 10:1 was set to be 0.4 ng/mL.

The accuracy and precision were determined for LLOQ, low, medium and high QC levels. For the intra-day assay, six replicates of each QC level were processed the same day. For the inter-day assay, each QC level was processed six-times on three consecutive days. Supplemental Table 1 summarizes the results of precisions and accuracies. All the values were within the acceptable criterion, demonstrating that the developed method was accurate and reliable.

The extraction recoveries of DHPPA were measured by comparing peak areas of QC samples spiked before extraction with those of post-spiked samples. The mean extraction recoveries were between 87.95 % and 94.19 % for DHPPA at three concentrations (Supplemental Table 1), indicating that the recoveries were acceptable. The matrix effect was evaluated by comparing the peak areas of the post-extracted spiked samples with those of the corresponding standard solutions. The results of IS-normalized matrix effects are also shown in Supplemental Table 1.

The stability of DHPPA in plasma was evaluated by analyzing QC samples at three concentrations in different conditions (room temperature, 24 h; three freeze-thaw cycles; -80 °C, 1 year). Stability values are summarized in Supplemental Table 2, showing that DHPPA were stable in plasma stored at room temperature for 24 h and -80 °C for 1 year. Moreover, no obvious reduction was observed after three freeze-thaw cycles.

SUPPLEMENTARY DATA

Supplementary Table 1. Precision, accuracy, recovery and matrix effect for the determination of DHPPA

Analyte	Spiked concentration (ng/mL)	Intra-day (n=6)			Inter-day (n=6)			Recovery (%, mean ± SD)	Matrix effect (%, mean ± SD)
		Measured concentration (ng/mL, mean ± SD)	RSD (%)	RE (%)	Measured concentration (ng/mL, mean ± SD)	RSD (%)	RE (%)		
DHPPA	0.4	0.41 ± 0.01	1.70	3.00	0.43 ± 0.01	2.55	8.00	--	--
	2	1.86 ± 0.08	4.03	-6.90	1.91 ± 0.04	2.04	4.55	88.26 ± 3.64	102.88 ± 4.92
	20	20.21 ± 0.62	3.09	1.04	19.82 ± 0.71	3.60	0.88	94.19 ± 8.13	103.69 ± 7.01
	40	40.52 ± 1.41	3.49	1.31	40.66 ± 1.59	3.92	1.64	87.95 ± 2.31	108.84 ± 1.48

Abbreviations: RSD: relative standard deviation; RE: relative error.

Supplementary Table 2. Stability of DHPPA in plasma (n = 5)

Analytes	Spiked concentration (ng/mL)	Room temperature for 24 h		Three freeze-thaw cycles		1 year, -80 °C	
		Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)	Precision (RSD, %)	Accuracy (RE, %)
DHPPA	2	2.05	-3.4	2.46	5.00	6.43	-2.40
	20	2.73	-10.7	3.72	4.2	4.81	4.69
	40	7.59	-10.3	4.11	-1.03	2.68	-0.47

Abbreviations: RSD: relative standard deviation; RE: relative error.

Supplementary Figure 1. MRM chromatograms of a blank sample (A, DHPPA; A', IS), a blank sample spiked with the analyte and IS (B, DHPPA; B', IS), and a plasma sample obtained from the participant (C, DHPPA; C', IS).