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Development and Validation of an LC-MS/MS Method for Determination of Damaurone D in Rat Plasma and its Application to Pharmacokinetic Study in Rats

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Abstract : Damaurone D belongs to the genus Rosa and is a traditional medicinal product used for the treatment of depression, inflammation, and infectious diseases. The purpose of this study was to develop a simple liquid chromatography-tandem mass spectrometry method for the detection of damaurone D in rat plasma and to demonstrate its application in pharmacokinetic studies. Damaurone D and berberine (internal standard) were extracted with acetonitrile using a protein precipitation method. Mass transition was monitored in multiple reaction monitoring mode at m/z 323.2→267.0 for damaurone D and m/z 336.1→320.0 for berberine in positive ion mode. Analytical validation was conducted by evaluating the specificity, linearity, accuracy, precision, matrix effect, extraction recovery, and stability. The calibration curves were linear over 2–1000 ng/mL. The intra- and inter-day precision and accuracy of quality control samples were 4.79–13.33% and 86.23–102.75%, respectively. The matrix effect and extraction recovery were 96.11–98.47% and 96.11–102.25%, respectively. In the pharmacokinetic study after intravenous administration of damaurone D at a dose of 3 mg/kg in rats, the area under the curve and clearance of damaurone D in rat plasma were 16750.26 ± 2676.10 min·ng/mL and 182.44 ± 31.36 mL/min/kg, respectively.

Key words : Damaurone D, LC-MS/MS, analytical method validation, pharmacokinetic study

Introduction

Members of the genus Rosa including Rosa damascene have been used as ingredients in traditional medicine formulations for treating depression, inflammation, and infectious diseases.[1,2] Damaurone D, a pharmacologically active component of Rosa damascene, has recently been reported as a potent anti-inflammatory agent, as demonstrated by its inhibitory effect on lipopolysaccharide-stimulated inducible nitric oxide synthase and cyclooxygenase-2 expression in murine macrophage RAW264.7 cells.[3,4] Han et al. reported a simple chemical synthesis process for damaurone D that allowed investigation of its pharmacological efficacy and biopharmaceutical properties through various in vitro and in vivo animal studies.[4] Despite the importance of analytical methods for preclinical and toxicokinetic studies, no analytical method has been reported for the quantitation of damaurone D. In this study, we developed a rapid, simple, and selective method for the quantitation of damaurone D in rat plasma by employing a protein precipitation technique and liquid chromatography with electrospray ionization-tandem mass spectrometry (LC-MS/MS). Our method was fully validated according to the guidelines of the US Food and Drug Administration (FDA) for bioanalytical methods in terms of linearity, specificity, accuracy, precision, extraction recovery, matrix effect, and stability.

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Experimental

Chemicals and reagents

Damaurone D (Figure 1) was synthesized with a purity of >99.0%, as confirmed by nuclear magnetic resonance and mass spectroscopy.[4] Berberine (internal standard, IS) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and water were purchased from Tedia (Fairfield, CT, USA). Dimethyl sulfoxide (DMSO) and formic acid were purchased from Sigma-Aldrich. All solvents and chemicals were of high-performance liquid chromatography and reagent grade.

Preparation of stock and working solutions

The stock solution was prepared by dissolving the damaurone D in DMSO at a concentration of 1 mg/mL. The working solutions of damaurone D for calibration and quality control (QC) were prepared by diluting the stock solution serially with acetonitrile at concentrations of 20, 50, 60, 100, 250, 1000, 1500, 5000, 7500, and 10000 ng/mL. The berberine solution was prepared at a concentration of 1 ng/mL in acetonitrile.

Preparation of calibration curve and QC samples

The samples for calibration were prepared by spiking a 5-μL aliquot of the working solution with a 45-μL aliquot of blank rat plasma, achieving final concentrations of 2, 5, 10, 25, 150, 500, and 1000 ng/mL. The QC samples were prepared in the same manner at concentrations of 6 (low QC), 100 (middle QC), 750 (high QC) ng/mL.

Sample preparation

A 50 μL aliquot of the calibration or QC samples was added to 200 μL of berberine solution (1 ng/mL in acetonitrile). The mixture was subjected to vigorous vortex for 15 min and centrifuged at 16,100 × g for 10 min. After centrifugation, a 5-μL aliquot of the supernatant was injected into the LC-MS/MS system.

Method Validation

The developed method was validated according to guidelines of the US FDA for bioanalytical methods based on specificity, linearity, accuracy, precision, extraction recovery, matrix effect, and stability. Blank plasma samples from six rats were used to assess the specificity. The signals from the six blank plasma samples were compared with those of corresponding lower limit of quantification (LLOQ) samples and IS. To determine the linearity, a calibration curve was generated in the concentration range of 2–1000 ng/mL. The linearity of the calibration curve was calculated from the peak response ratio of damaurone D to berberine (IS) with the weight-adjusted method ($1/x^2$). Six replicates were prepared at three QC levels (low, middle, and high QC) to assess intra-day precision and accuracy, and two replicates were prepared at the three QC levels to assess inter-day precision and accuracy during five consecutive days. The peak areas of damaurone D in the QC samples were compared with those in the samples prepared by spiking the extracted blank plasma samples with corresponding concentrations of damaurone D to assess the extraction recovery. The matrix effect was evaluated by comparing the peak areas of damaurone D in samples prepared by spiking the extracted blank plasma samples with damaurone D at the three QC concentrations to those of damaurone D in neat solution samples with corresponding concentrations of damaurone D. Extraction recovery and matrix effect of the IS were also determined in the same way as those of damaurone D. The stability of damaurone D in rat plasma was examined at the three levels of QC exposed to different conditions. Bench-top stability was calculated by comparing QC samples that were stored for 22 h at room temperature with untreated QC samples. Freeze-thaw stability was analyzed by comparing QC samples that underwent three freeze-thaw cycles with untreated QC samples. Autosampler stability was evaluated by comparing the extracted QC samples maintained in an autosampler at 6°C for 12 h with untreated QC samples.

Pharmacokinetic study

All animal procedures were approved by the Animal Care and Use Committee of the Kyungpook National University (Permission no. 2016-0019). Male Sprague-Dawley rats (8 weeks, 250-270 g) were purchased from SAMTAKO (Osan, Kyunggi-do, Korea). The rats were housed in a 12/12-h light/dark cycle and food and water were supplied ad libitum for one week prior to the animal experiment. Damaurone D was intravenously administered to rats at a dose of 3 mg/kg. Blood samples were collected from the femoral artery 0, 2, 5, 15, 30, 60, 120, 180, 240, 360, and 480 min following damaurone D injection. The blood samples were centrifuged at 16,100 × g for 10 min to separate the plasma and 50-μL aliquots of plasma samples were stored at -80°C until analysis.

LC-MS/MS analysis of Damaurone D

Damaurone D in rat plasma samples was detected by an Agilent 6430 Triple Quadrupole LC-MS/MS system (Agilent, Wilmington, DE, USA). The isocratic mobile phase consisting of a mixture of water and acetonitrile (30:70, v/v) containing 0.1% formic acid was used at a flow rate of 0.20 mL/min. Separation was performed on a Synergi Polar RP column (150 × 2 mm, particle size of 4 μm, Pheonomenex, Torrance, CA, USA). Mass transition was monitored in multiple reaction monitoring (MRM) mode at m/z 323.2→267.0 for damaurone D and m/z 336.1→320.0 for berberine (IS) in positive ion mode with a collision energy of 10–15 eV. The analytical data were quantified using MassHunter (version B.06.00, Agilent, Wilmington, DE, USA).

Data Analysis

Pharmacokinetic parameters were assessed by non-compartmental analysis (WinNonlin 2.0; Pharsight, Mountain View, CA, USA). The area under the plasma concentration-time curve from zero to infinity was calculated by the trapezoidal extrapolation method. All data are expressed as the mean ± standard deviation (SD)

Results and discussion

Determination of LC-MS/MS conditions

To optimize the MS conditions for damaurone D and berberine (IS), each compound was injected directly into

the MS/MS system. Damaurone D and the IS showed optimal ionization in positive ionization mode. MRM transition of damaurone D was selected from the precursor ion ($[M+H]^+$, m/z 323.2) and the most frequent product ion (m/z 267.0). MRM transition of the IS was selected from the precursor ion ($[M+H]^+$, m/z 336.1) and the most frequent product ion (m/z 320.0).[5] Figure 2 shows the structure and product ion scan spectra of damaurone D and the IS. The optimal MRM transition m/z ratios, fragmentor voltage, and collision energy (CE) for damaurone D and the IS were obtained and further analyses were performed at conditions of m/z 323.2→267.0 for damaurone D and m/z 336.1→320.0 for the IS at a fragmentor voltage of 135 V and CE of 20 or 25 eV, respectively.

LC conditions and various analytical columns were evaluated in terms of signal response, peak shape, and retention time. Damaurone D showed higher abundance in the mobile phase with acetonitrile rather than methanol as an organic solvent. The Synergi Polar RP column showed good signal response and peak shape among the columns tested. In the Synergi Polar RP column, the signal response and retention time of damaurone D were decreased with the increase in the concentration of acetonitrile in the mobile phase. Finally, the mixture of water and acetonitrile (30:70, v/v) containing 0.1% formic acid was chosen as the optimal composition of the mobile phase considering the run time and LLOQ.

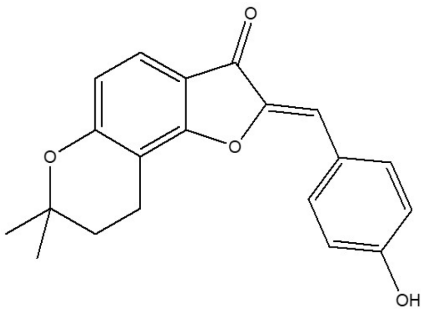

Figure 1. Structure of damaurone D.

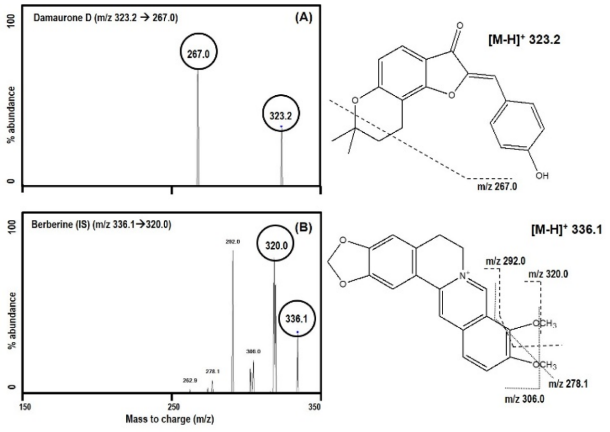

Figure 2. Product ion spectra of damaurone D (A) and berberine (B).

Analytical method validation

Figure 3 shows the typical MRM chromatograms of the double blank, zero blank, LLOQ (2 ng/mL), and plasma samples after intravenous administration of damaurone D. The retention times of damaurone D and the IS were 2.63 and 3.30 min, respectively. There was no significant matrix interference at the retention times of damaurone D and the IS in the double blank samples compared with the LLOQ samples (Figure 3). The calibration standard curves showed good linearity over the concentration range of 2–1000 ng/mL ($r^2 > 0.998$).

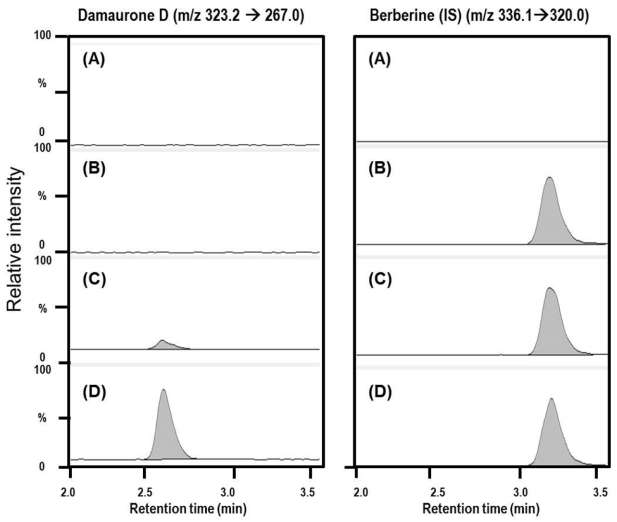

Figure 3. Representative MRM chromatograms of damaurone D (left panel) and of berberine (right panel) in (A) double blank sample, (B) zero blank sample, (C) LLOQ sample (2 ng/mL), and (D) plasma sample at 30 min following IV injection of damaurone D.

Table 1. Intra- and inter- day precision and accuracy of damaurone D in rat plasma.

	Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Precision (%)	Accuracy (%)
Intra-day (n=6)	6	5.88 ± 0.78	13.33	98.08
	100	86.23 ± 4.13	4.79	86.23
	750	719.23 ± 46.08	6.41	95.90
Inter-day (n=5)	6	5.99 ± 0.38	6.40	99.76
	100	91.88 ± 6.70	7.29	91.88
	750	770.64 ± 47.98	6.23	102.75

Data represented as mean ± SD from five or six independent experiments.

Table 2. Extraction recoveries and matrix effects for the determination of damaurone D and of berberine (IS).

Nominal concentration (ng/mL)	Extraction recovery (%)	CV (%)	Matrix effect (%)	CV (%)
Damaurone D				
6	102.25 ± 8.22	8.04	96.11 ± 6.31	6.57
100	97.94 ± 8.14	8.32	97.77 ± 2.19	2.23
750	95.30 ± 2.81	2.95	98.47 ± 2.17	2.20
Berberine				
1	105.05 ± 3.33	3.17	99.23 ± 2.71	2.73

Data represented as mean ± SD from six independent experiments.

Table 3. Stability of damaurone D in rat plasma.

Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Precision (%)	Accuracy (%)
Bench-top stability			
6	6.04 ± 0.48	8.01	100.61
100	97.41 ± 5.67	5.82	97.41
750	757.21 ± 36.20	4.78	100.96
Freeze-thaw stability			
6	5.73 ± 0.56	9.77	95.50
100	102.63 ± 2.43	2.36	102.63
750	808.13 ± 57.38	7.10	107.75
Autosampler stability			
6	6.22 ± 0.23	3.76	103.67
100	96.91 ± 3.08	3.18	96.91
750	791.34 ± 13.14	1.66	105.51

Data represented as mean ± SD from six independent experiments.

The intra- and inter-day precision and accuracy for damaurone D are summarized in Table 1. The intra-day precision ranged from 4.79–13.33%, and the intra-day accuracy ranged from 86.23–98.08%. The inter-day precision ranged from 6.23–7.29%, and the intra-day accuracy ranged from 91.88–102.75%. The results of extraction recovery and matrix effect are shown in Table 2. The extraction recovery for damaurone D was found to be high and reproducible in the range of 95.30–102.25% with a coefficient of variation (CV) of lower than 8.32%. The matrix effect ranged from 96.11–98.47% with a CV of lower than 6.57%. These results indicated that the protein precipitation method employed in this study enabled simple and efficient extraction of damaurone D from rat plasma. In addition, the bench-top, freeze-thaw, and autosampler stability of damaurone D were evaluated. As shown in Table 3, damaurone D was stable for up to 22 h at bench top at room temperature, for 12 h in an autosampler at 6°C, and over three freeze-thaw cycles.

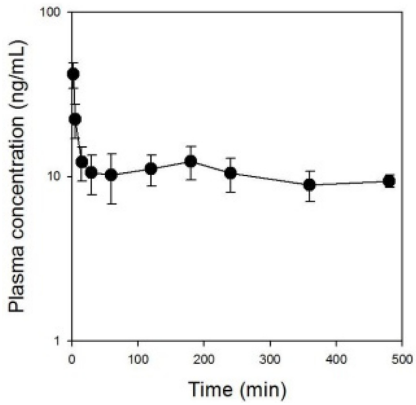

Figure 4. Plasma concentration-time profile of damaurone D after intravenous administration of damaurone D at a single dose of 3 mg/kg. Each data represents the mean ± SD from three different rats.

Table 4. Pharmacokinetic parameters of damaurone D following intravenous administration of damaurone D in rats.

Parameters	
AUC (ng·min/mL)	16750.26 ± 2676.10
CL(mL/min/kg)	182.44 ± 31.36
$V_{d,ss}$ (L/kg)	21.87 ± 5.58

Each data represents the mean ± SD from three rats.

Pharmacokinetic profiles of damaurone D

The mean plasma concentration-time profiles of damaurone D after a single intravenous administration are shown in Figure 4. The pharmacokinetic parameters of damaurone D are listed in Table 4. The plasma concentration of damaurone D declined sharply within 15 min and was maintained until the last sampling time point (480 min) in this study. This suggests that damaurone D showed a multi-exponential elimination pattern with a fast distribution phase and stable elimination phase, which could be attributed to the large distribution volume of this compound, although the underlying mechanism needs to be further investigated. The area under the curve was calculated to be 16750.26 ± 2676.10 ng·min/mL. The clearance and volume of distribution at steady-state ($V_{d,ss}$) with intravenous administration were calculated as 182.44 ± 31.36 mL/min/kg and 21.87 ± 5.58 L/kg, respectively.

Conclusions

An analytical method for evaluating damaurone D was developed for the first time using LC-MS/MS, and the method was successfully validated according to the

guidelines of the US FDA. The present method for the detection of damaurone D showed good specificity and linearity with acceptable matrix effect and satisfied the acceptance criteria in terms of accuracy, precision, extraction recovery, and stability. Moreover, the method required the use of a small volume of plasma (50 μL) and involved simple sample preparation steps (protein precipitation). The developed method could be useful in preclinical pharmacokinetic studies of damaurone D.

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