HPLC Determination and PharmacoKinetic Study of Homoeriodictyol-7-O-ß-D-glucopyranoside in Rat Plasma and Tissues

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Received June 7, 2006; accepted January 17, 2007

Homoeriodictyol-7-O-ß-D-glucopyranoside (HEDT-Glu) was isolated from Viscum coloratum and identified by MS, 1H- and 13C-NMR. A HPLC method was developed for determination of HEDT-Glu in rat plasma and tissues. All biological samples were pretreated by protein precipitation with acetone. Vanillin was selected as internal standard. The mobile phase consisted of methanol–water–glacial acetic acid (45 : 55 : 0.5, v/v/v). Good linearity were observed over the concentration ranges of 0.1—200.0 µg·ml⁻¹ in rat plasma and 0.05—5.0 µg·ml⁻¹ in tissues. Both intra- and inter-day precisions of HEDT-Glu, expressed as the relative standard deviation, were less than 13.1%. Accuracy, expressed as the relative error, ranged from —0.8 to 5.4% in plasma and from —5.6 to 9.4% in tissues. The mean extraction recovery of HEDT-Glu was above 73.17% in biological samples. The described assay method was successfully applied to the pre-clinical pharmacokinetic study of HEDT-Glu. After intravenous administration of HEDT-Glu to rat, AUC₁/₂, and CLₑ were 16.04±3.19 µg·h·ml⁻¹ and 0.85±0.17 l·kg⁻¹·h⁻¹, respectively. T₁/₂,α and t₁/₂,ß were 0.06±0.01 h and 1.27±0.31 h, respectively. HEDT-Glu was cleared from the blood and mainly distributed to the liver and small intestine.

Key words  homoeriodictyol-7-O-ß-D-glucopyranoside; Viscum coloratum; rat plasma; tissue; HPLC determination; pharmacokinetics

MATERIALS AND METHODS

Chemicals  Viscum coloratum was obtained from Tianyitang traditional Chinese medicine store (Shenyang, China). HEDT-Glu was isolated and purified in our laboratory. Methanol (HPLC grade) was purchased from Concord Tech Reagent Company (Tianjin, China). All other reagents were of analytical grade from Shenyang Damao Chemical Reagent Factory (Shenyang, China). Distilled water, prepared from demineralized water, was used throughout the study. Vanillin (Internal standard, IS) was obtained from E. Merck (Darmstadt, Germany). Polyamide was purchased from Taizhou sijia Biochemistry and Plastic factory (Taizhou, China).

Animals  Male and female pathogen-free Wistar rats (200—240 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University and the protocol was approved by the Animal Ethics Committee of this institution. The rats were fed standard laboratory food and water for at least 3 days before the experiments.

Instruments and Chromatographic Conditions  The chromatographic system (Shimadzu, Kyoto, Japan) consisted of a pump (LC-10ATVP), an UV detector (LC-10A VP), a column oven (TC-100) and a LC-Workstation (Anastar). Chromatography was performed on a Diamonsil C₁₈ column (200 mm×4.6 mm i.d., particle 5 µm, DKma, Beijing, China). A mobile phase consisted of methanol–water–glacial acetic acid (45 : 55 : 0.5, v/v/v). The flow-rate was 1.0 ml·min⁻¹. Ultraviolet detection wavelength was set at 284 nm and the column temperature was kept at 30°C. Identification of HEDT-Glu was carried out by MS (Finnigan TSQ), 1H- and 13C-NMR spectra (Bruker AVANCE-300).

Isolation and Purification of HEDT-Glu  Branches and leaves of Viscum coloratum were splintered. After removal of lipophilic extracts using solvent extraction with ligarine,
the hydrophilic extractives were extracted with n-butanol.\(^\text{15}\) HEDT-Glu (96—98\%) can then be obtained by chromatography on polyamide column and by crystallization from the methanol. The substance was submitted to instrumental analysis using \(^1\text{H}, \ ^{13}\text{C}-\text{NMR} \) and MS.

**Preparation of Standard Solutions** Stock solution of HEDT-Glu (1.0 mg·ml\(^{-1}\)) and IS (500.0 μg·ml\(^{-1}\)) were prepared in methanol. The solution of HEDT-Glu was then serially diluted with methanol to achieve standard working solutions at the concentrations of 0.1, 0.5, 2.0, 10.0, 50.0, 100.0 and 200.0 μg·ml\(^{-1}\) for plasma and at the concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, 2.5 and 5.0 μg·ml\(^{-1}\) for tissues. Quality-control (QC) solution for plasma (0.5, 10.0, 160.0 μg·ml\(^{-1}\)) and for tissue (0.1, 0.5, 2.0 μg·ml\(^{-1}\)) were independently diluted. IS working solution for plasma (25.0 μg·ml\(^{-1}\)) and for tissues (10.0 μg·ml\(^{-1}\)) were prepared by diluting the 500.0 μg·ml\(^{-1}\) stock standard solution in methanol, respectively.

**Sample Preparation** Plasma samples of 100 μl (tissues samples of 200 μl) from rats were transferred to tubes which were added 500 μl (1000 μl) aceton and 50 μl internal standard. This mixture was vortex-mixed for 1 min. After centrifuging for 3 min at 12000 rpm, the supernatants were evaporated to dryness under a stream of nitrogen at 40 °C. The residues were reconstituted in 100 μl mobile phase, vortex-mixed for 20 s, and then centrifuged for 3 min at 12000 rpm. The supernatants of 10 μl were injected into the HPLC system for analysis.

**Method Validation** Calibration, Precision, and Accuracy: Samples spiked with HEDT-Glu at seven concentrations were prepared in duplicate and analyzed in three separate analytical runs. Calibration curves were constructed using weighted (\(w = 1/x^2\)) least-squares linear regression analysis of the observed peak area ratios of HEDT-Glu and IS versus the respective standard concentrations.\(^\text{16}\) The unknown samples concentrations were calculated from the linear regression equation of the peak area ratio against concentrations of the calibration curve. The QC samples at low, medium and high levels and the limit of quantification (LOQ) sample were analyzed to access the accuracy and precision of the proposed method. In order to determine intra-day and precision, 6 duplicates were sampled on the same day. Inter-day accuracy and precision were determined over a period of 3 days with 6 duplicates per day (\(n = 18\)). The precision was evaluated as the relative standard deviation (RSD), while the accuracy was expressed as the relative error (RE).\(^\text{17,18}\)

Extraction Recovery and Analytical Stability: The extraction recoveries were determined at three concentration levels by comparing the analyte peak areas, obtained from the QC (\(n = 6\)) after extraction, with those obtained from the corresponding unextracted reference standards prepared at the same concentrations. The stability of HEDT-Glu in rat plasma and tissues were investigated under a variety of storage conditions: performing three cycles of freeze (−20 °C)—thaw (room temperature), 24 h storage at room temperature and under −20 °C freezer at least one month.

**Application of the Method** Plasma Samples: Each rat (\(n = 6\)) was fasted for 12 h, with free access to water, during the experiments and administered injection of 13.2 mg·kg\(^{-1}\) HEDT-Glu via the tail vein. The blood samples were collected at 0, 0.016, 0.083, 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, and 5.0 h after intravenous (i.v.) administration and centrifuged to obtain plasma.

Tissue Samples: The rats were decapitated 0.083, 0.25 and 1.0 h after i.v. administration of HEDT-Glu. The brain, liver, kidney, spleen, pancreas, lung, heart, skeletal muscle, stomach, small intestine, and fat were immediately removed, blotted onto filter papers and weighted. The tissues were minced in saline and homogenized with homogenizer. The homogenate were stored at −20 °C.

The plasma (tissues) concentrations of HEDT-Glu at different time were expressed as mean±S.D. All data of plasma concentrations were subsequently processed by the computer program 3p97 (Practical Pharmacokinetic Program, China) to determine the compartment models and pharmacokinetic parameters.

**RESULTS AND DISCUSSION**

**Structural Identification of HEDT-Glu** HEDT-Glu was a whitish crystal and its mp was 158—160 °C. HCl–Mg, Molish and NaBH\(_4\) reactions were all positive, indicating a glycoside of flavanone. MS (m/z): 177, 274, 302 (aglycone), 464 (M\(^+\)). The date given by \(^1\text{H}-\text{NMR} \) (DMSO) \(\delta \) ppm were 2.76 (1H, dd, \(J = 4, 18\) Hz, \(C_1\) cis-H); 3.22—3.47 (\(m\), glycoside of H, OH and \(C_3\) trans-H); 5.04 (1H, m, indicating glycoside H-1), 3.79 (3H, s, \(C_2\)-OCH\(_3\)); 5.49 (1H, dd, \(J = 4, 12\) Hz, \(C_2\)-H); 6.17 (2H, s, \(C_6\)-H); 6.90 (1H, d, \(J = 8.5\) Hz, \(C_2\)-H); 6.92 (1H, dd, \(J = 8.5, 2\) Hz, \(C_3\)-H); 7.11 (1H, d, \(J = 2\) Hz, \(C_3\)-H); 9.18 (1H, s, \(C_5\)-OH); 12.07 (1H, s, \(C_1\)-OH).\(^\text{13}\text{C}-\text{NMR} \) (DMSO) \(\delta \) ppm: 156.5 (C-2), 138.5 (C-3), 178.2 (C-4), 161.0 (C-5), 98.0 (C-6), 165.3 (C-7), 92.7 (C-8), 155.4 (C-9), 148.7 (C-4\(^1\)), 115.0 (C-5\(^1\)), 123.3 (C-6\(^1\)), 59.9 (OCH\(_3\)), 56.3 (OCH\(_3\)), 56.0 (OCH\(_3\)), 99.6 (C-1\(^1\)), 73.2 (C-2\(^2\)), 77.2 (C-3\(^3\)), 69.7 (C-4\(^4\)), 77.0(C-5\(^5\)), 60.7(C-6\(^6\)). Based on these data, the compound was identified as HEDT-Glu with formula of \(C_{22}H_{32}O_{11}\) and molecular weight of 464, which were consistent with the standard data.\(^\text{19}\)

**Validation of Chromatographic Methods** Assay Specificity and LOQ: The specificity of method was demonstrated by comparing chromatograms obtained from plasma (tissues) samples of rats, each as a blank and a spiked sample. Figures 2 and 3 show that the peaks of compound in plasma and tissues were not interfered by endogenous substances, respectively. The retention time of internal standard and HEDT-Glu were approximately 6.80 and 9.90 min, respectively. The LOQ was 1 ng in plasma samples and was 0.5 ng in tissues samples.

Calibration, Precision, and Accuracy: Good linearity were observed over the concentration range of 0.1—200.0 μg·ml\(^{-1}\) in rat plasma and 0.05—5.0 μg·ml\(^{-1}\) in tissues. For the samples prepared in plasma, kidney, spleen, liver, and small intestine, the mean regression equation were \(y = 0.040x + 0.0035 \) (\(r = 0.9965\)); \(y = 0.112x - 0.0148 \) (\(r = 0.9981\)); \(y = 0.1103x - 0.0027 \) (\(r = 0.9945\)); \(y = 0.108x + 0.0017 \) (\(r = 0.9974\)); \(y = 0.1102x - 0.0258 \) (\(r = 0.9932\)), respectively, where \(y \) is the peak area ratio and \(x \) is the concentration. In this assay, the intra- and inter-day precision in plasma ranged from 3.4 to 7.5% and from 1.9 to 9.7%, respectively. The results of tissues never exceeded 13.1%. The accuracy
ranged from −0.81 to 5.42% in plasma and from −5.6 to 9.4% in tissues. Related data are given in Table 1. These data suggested that the method was accurate and reproducible for the determination of HEDT-Glu in rat plasma and tissues.

Extraction Recovery and Analyte Stability: The mean extraction recoveries of HEDT-Glu was above 73.17% in biological samples. The extraction recovery of IS recovery was found 82.05.8%. Related data are given in Table 1. The QC samples prepared in rat plasma and tissues, after undergoing three freeze-thaw cycles, showed no significant degradation. In extracts, these compounds were stable for up to 24 h at ambient temperature. Also, these were no significant difference in plasma and tissues at −20 °C for up to 1 month. Stock solutions of the compounds in methanol were stable for up to 45 days.

Applications of the Analytical Method in Pharmacokinetic Studies After i.v. injection of 13.2 mg·kg⁻¹ HEDT-Glu to rats, plasma concentrations of HEDT-Glu were determined by the described HPLC. Figure 4 shows the mean plasma concentration–time curve of HEDT-Glu (n=6).
**CONCLUSIONS**

For the first time, a simple and reliable HPLC method to determine HEDT-Glu levels in rat plasma and tissues was established and validated, which had been successfully applied to the pharmacokinetics studies. The developed assay showed acceptable precision, accuracy, linearity, stability and specificity. It can be applied to extensive human pharmacokinetic studies as well.

**Acknowledgements** This study was supported in part by State Pharmacopeia’s Committee of People’s Republic of China.

**REFERENCES**