Validation of (S)-zaltoprofen Assay in Rat Plasma by HPLC and Its Application in Pharmacokinetics Study
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Purpose
(S)-zaltoprofen ((S)-ZPF) is a non-steroidal anti-inflammatory drug, which has analgesics, antipyretics, and anti-inflammatory activities. Some previous studies showed that (S)-ZPF exerted better anti-inflammatory and analgesic activities than racemic zaltoprofen in rodents and also expressed a higher bioavailability compared to (R)-zaltoprofen. However, to our knowledge, no studies have been reported on validation of quantification method of pure (S)-ZPF in rat plasma as well as an evaluation of bioavailability of (S)-ZPF in rats. Hence, this study focused on establishing a validated method of (S)-ZPF in rat plasma using HPLC and also evaluating the kinetic profiles of (S)-ZPF after oral and intravenous route administration.

Methods
The liquid-liquid extraction of (S)-ZPF and ketoprofen (IS) from rat plasma (0.125 mL) into the mixture of acetonitrile and dichloromethane (1:6, v/v) was employed. The organic layer was separated and evaporated under a gentle stream of nitrogen at 40 °C. The residue was reconstituted in the according mobile phase or MeOH and injected onto HPLC. Two different HPLC columns including a reversed column C18 (with mobile phase containing acetonitrile-distilled water (55:45, v/v) at a flow rate of 1.2 mL/min.) and a chiral column Chiralcel OJ-H (with mobile phase containing n-hexane-2-propanol-trifluoroacetic acid (90:10:0.1, v/v/v) at a flow rate of 0.8 mL/min) were used. The eluates were monitored using an ultraviolet (UV) detector set at 240 nm. To look into in vivo study of (S)-ZPF in rats, six SD rats were employed according to the 'Guiding Principles in the Use of Animals in Toxicology' adopted by the Society of Toxicology (USA) and the experimental protocols were approved by the Animal Care Committee of Chungnam National University. The suspension of (S)-ZPF using in oral route was prepared in the mixture of 5% (v/v) DMSO containing 20% (w/v) PEG 400 in a 5% (w/v) glucose solution. To prepare of solution applied to intravenous administration, 5 mg of (S)-ZPF was dissolved completely in ethanol and then the given solution was diluted 10 times with 20 % (w/v) PEG 400 solution. Blood samples (0.8 mL) were collected from the orbital vein at desirable time points. Within 30 min following blood withdrawal, the samples were centrifuged at 15,000 rpm for 15 min at 4 °C. The plasma was collected, labeled and stored at -80 °C prior to application of above analytical method.

Results
The validation included assessment of linearity, recovery, accuracy, precision, and stability in rat plasma. Under the mentioned reversed HPLC conditions, (S)-ZPF and ketoprofen were detected separately at 4.16 and 2.95 minutes, respectively. Response was linear over the calibration ranges of 0.1-75 μg/mL with R² of 0.9998. The lower limit of quantification was 1.92 μg/mL, and the percent coefficient of variation was within 3%. In addition, (S)-ZPF was stable under various store conditions such as three cycles of freezing-thawing and 4 °C for 24 hr. The results also revealed that there was not any transformation of (S)-ZPF into (R)-zaltoprofen and absolute bioavailability of (S)-ZPF through oral administration was 71.89 ± 3.43%. In oral administration, (S)-zaltoprofen showed the dramatic longer half-life (t½) of 9.34 ± 0.89 h compared to the half-life in intravenous administration at 6.04 ± 0.85 h.

Conclusion
The assay developed is linear, accurate, precise and reproducible for the analysis of (S)-zaltoprofen in rat plasma. The method can be practically adopted in the characterization of (S)-ZPF pharmacokinetics after single oral and intravenous dose.