Quantification of Tangeretin in Rat Plasma: Application to a Pharmacokinetic Study
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Purpose
Tangeretin (TAN) is dietary polymethoxylated flavone with a wide range of health-promoting activities as a functional food ingredient. It is one of the main polymethoxylated flavones (PMFs) in Citrus reticulate (tangerine) and Citrus sinensis (sweet orange). It was shown in previous studies that tangeretin possesses many pharmacological effects such as antimicrobial effect; anticancer effect; anti-diabetic activity; chondroprotective effect and anti-inflammatory activity. To be able to evaluate the health promoting activities of TAN, first its absolute oral bioavailability (F) needs to be determined. The available reports showed that pharmacokinetic profiles of TAN are not well elucidated yet. No absolute oral bioavailability has been reported up to date. Consequently, investigating its pharmacokinetic profiles is warranted to be able to further formulate it into a nutraceutical/functional food ingredient and this is the purpose of our current study.

Methods
In this study, a sensitive HPLC-UV method was developed and validated to quantitate TAN in rat plasma. Pharmacokinetic profiles of TAN were examined in Sprague-Dawley rats after single intravenous dosing in one group of rats (n=5) and oral dosing in 2 groups of rats, one group for oral solution (n=6) and the other group for oral suspension (n=5). The oral dosing formulations were chosen to be in both solution and suspension forms so as to determine the effect of the aqueous solubility of TAN on its bioavailability. In this pharmacokinetic study, animals were maintained on a 12-h light/dark cycle with free accesses to food and water. On the day before the pharmacokinetic study, a polyethylene tube was placed into the right jugular vein under isoflurane anesthesia. This cannula was used for intravenous drug dosing and blood sampling. Plasma samples were collected from the 3 groups up to 12 hours and were stored at -80 degrees Celsius until further analysis. Clean up of samples was done by simple protein precipitation methods.

Results
This HPLC method was validated by examining its selectivity, sensitivity, accuracy, precision, and the stability profiles of TAN. Under our chromatographic conditions, TAN (peak 1) and IS (peak 2) were completely separated and eluted at ~ 7.4 and 8.7 min respectively (Figure 1 A: No significant interference peaks were observed in the chromatograms of either blank plasma samples (n = 6) or pre-dosing plasma samples (n = 16); a typical chromatogram of a pre-dosing sample is shown in Figure 1 B). Moreover, TAN and IS were well separated from the metabolites (peaks 3 and 4) in the chromatograms acquired from post-dosing samples (Figure 1 C & D). The lower LOQ of TAN was found to be 15 ng/ml. The calibration curves were linear (R² > 0.999). This HPLC method had good precision as the intra-day or inter-day RSDs were all less than 10%.

Upon single intravenous administration (10 mg/kg), TAN displayed a moderate apparent volume of distribution (Vc = 4.20 ± 0.82 L/kg), a rapid clearance (Cl = 94.1 ± 20.2 ml/min/kg) and a moderate terminal elimination half-life (1/2 ß = 166 ± 42 min). At 12 h post-administration, TAN level dropped below lower LOQ (< 15 ng/ml). After single oral administration (50 mg/kg) in a suspension form, its absolute oral bioavailability (F) was only ~2% while when administered in an oral solution form was about ~6.5%. This 4 folds increase in (F) when TAN was dosed in a solution form shows that its low aqueous solubility acts as a barrier for its oral bioavailability. Overcoming this absorption barrier with a solubility enhancing excipient such as RM-β-CD has significantly enhanced the bioavailability, although it's still low. Metabolite peaks were observed in the chromatograms of almost all the collected samples (Figure 1C & 1D), which might be the reason TAN showed a relatively low oral bioavailability even when RM-βCD was used as an excipient to enhance its aqueous solubility.

Conclusion
As TAN was orally bioavailable, its further development as a nutraceutical/functional food ingredient is warranted. According to the authors’ knowledge, this is the first report of absolute oral bioavailability of TAN. Findings from this study warrant further exploration of TAN as a potential nutraceutical/functional food ingredient since it was found to be orally bioavailable; however, a potential set back might be its possible metabolism.

![Figure 1](image-url)