Quantification of 5,7-Dimethoxyflavone in Mouse Plasma Using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and Its Application to a Pharmacokinetic Study

D. Bei, G. An
University of Florida

**Purpose**

5,7-Dimethoxyflavone (5,7-DMF) is a natural flavonoid that is abundant in many herbal plants, such as Kaempferia Parviflora (also known as Thai Ginseng) and Piper Caninum. 5,7-DMF has been reported to have many beneficial pharmacological effects, including anti-inflammatory and chemopreventive properties. Recently, 5,7-DMF was also found to have potent inhibitory effects on efflux transporters and may represent a very promising chemosensitizing agent to reverse the efflux transporter-mediated multi-drug resistance. However, to date 5,7-DMF was evaluated mainly in vitro and the information related to its pharmacokinetics (PK) in vivo is very limited. In addition, current available quantification methods of DMF all lack sufficient sensitivity (LLOQ> 800 ng/mL). The purposes of our study are to establish a sensitive quantification method of DMF using LC-MS/MS assay and evaluate the PK profile of DMF in mouse.

**Methods**

Sample preparations included working solution preparation by serial dilution, liquid-liquid extraction with ethyl acetate and reconstitution. This method was fully validated and all of the fundamental parameters in method validation, including accuracy, precision, sensitivity, selectivity, recovery and stability were evaluated thoroughly in mouse plasma. In the PK study, 25 mg/kg of DMF was administered intravenously to BALB/c mice and the plasma samples were collected up to 34 hours. The plasma concentration-time data was analyzed by a non-compartmental method using Phoenix 1.3.

**Results**

The calibration curve covered 0-1000 ng/mL with the lower limit of quantification (LLOQ) at 2 ng/mL. The inter-day and intra-day precision and accuracy were all within ±15% criteria. The matrix effect and recovery yield were 86.8-99.8% and 87.4-99.8%, respectively for DMF at concentrations 2, 5, 50 and 500 ng/mL. DMF was stable in up to 3 cycle of freeze-thaw and in -80°C freezer for 2 weeks. Mouse plasma level of DMF was detectable up to 34 hours; terminal half-life t1/2 is 0.34 hr-1; Vz is 7.74 L/kg; CL is 2.61 L/hr/kg; and AUCinf is 9.58E+03 hr*ng/mL.

**Conclusion**

A fast, accurate, sensitive and selective quantification method of DMF was established to warrant further PK study in mouse. The developed method was successfully applied to a PK study of 5,7-DMF following i.v administration of 5,7-DMF in mice.