

## High Performance Liquid Chromatographic Analysis of Isoflavones in KUNBO

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Phytoestrogen has been used as supplement in order to treat osteoporosis. The representative phytoestrogen, isoflavones, are daidzein, genistein and formononetin which were present highly in legumes. We have studied the quantitative analysis of isoflavones in KUNBO by HPLC. KUNBO, a mixed herbal extract including Astragali Radix and Rhynchosiae nulubilis Semen (Leguminosae), etc., is a nutraceutical candidate for type I osteoporosis. Column used in HPLC was LUNA 5 $\mu$  C18 (250  $\times$ 4.6 mm) (Phenomenex Co., Torrance, CA, U.S.A.). Mobile phase used in HPLC was 5mM NaH<sub>2</sub>PO<sub>4</sub> (pH 4.60) : MeOH (1:1) (Flow rate : 1.30 ml/min). We are measured at 260nm (UV/VIS detector). The content of aglycone daidzein was 20.04  $\pm$ 0.40 mg/kg, and total daidzein was 417.82  $\pm$ 8.71 mg/kg. The content of aglycone genistein was 14.80  $\pm$ 0.09 mg/kg and total genistein 148.39  $\pm$ 1.85 mg/kg. The content of aglycone formononetin was 21.84  $\pm$ 0.11 mg/kg and total formononetin was 143.86  $\pm$ 6.01 mg/kg. The retention times were 12.30 min for daidzein, 19.73 min for genistein and 37.99 min for formononetin. Thus, we have established the QC standard for KUNBO as the results. (Supported partially by a grant, # 02-PJ1-PG11-VN04-SV04-0004, from Health Technology Planning & Evaluation Board, Korea)

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## Determination of a Novel Antiangiogenic Agent KR-31831 in Rat Plasma by Liquid Chromatography-Tandem Mass Spectrometry

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A liquid chromatography-tandem mass spectrometric (LC/MS/MS) method was developed for the determination of a new anti-angiogenic drug KR-31831 in rat plasma. KR-31831 and internal standard, KR-31543 were extracted from rat plasma with dichloromethane at basic pH. A reverse-phase LC separation was performed on Luna C8 column with the mixture of acetonitrile-ammonium formate (10 mM, pH 4.5) (67:33, v/v) as mobile phase. The detection of analytes was performed using an electrospray ionization tandem mass spectrometry in the multiple-reaction-monitoring mode. The standard curve was linear ( $r = 0.999$ ) over the concentration range of 1.0-500 ng/ml. The coefficient of variation of intra- and inter-assay ranged from 0.8-3.9% and 1.4-3.9%, respectively. The recoveries of KR-31831 ranged from 80.9 to 86.7%, with that of KR-31543 (internal standard) being 99.2  $\pm$  2.7 %. The lower limits of quantification for KR-31831 was 1.0 ng/ml using 100 ml plasma sample. This method was applied to the pharmacokinetic study of KR-31831 in rats.

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## Determination of Acetyl-L-carnitine in human plasma by LC-ESI/MS/MS

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Acetyl-L-carnitine, a physiological component of the L-carnitine family, has been proposed for treating Alzheimer's disease in pharmacological doses. Acetyl-L-carnitine and d3-acetylcarnitine (internal standard) were analyzed by electrospray ionization / tandem mass spectrometry (ESI/MS/MS) after derivatization to their butylesters through treatment with butanolic hydrogen chloride. Acetyl-L-carnitine produced a protonated precursor ion at  $m/z$  260 and a corresponding product ion of  $m/z$  85. Analytes were separated on a Capcell Pak C18 (2.0X150mm, 5 mm). The mobile phase was 40% acetonitrile with flow rate at 200 mL/min. Detection of acetyl-L-carnitine in human was accurate and precise, with a limit of quantitation of 0.1  $\mu$ g/mL. The calibration curves for acetyl-L-carnitine was linear in a concentration range of 0.1 ~ 20  $\mu$ g/mL. This method has been successfully applied to a study of acetyl-L-carnitine in human plasma.