

Research Article

Pharmacokinetics of Daidzein 4'-Phenylsulfonyl Ester and Its Active Metabolite Daidzein Assayed by HPLC Method in Rats After Intravenous Administration and Oral Administration

Xue-Mei ZHU and Jiang-Ning HU*

State Key Laboratory of Food Science and Technology, Nanchang University, PR China

***Corresponding author:** Jiang-Ning HU, State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, PR China, Tel: +86 7918304402; E-mail: hujiangning@ncu.edu.cn

Citation: Xue-Mei ZHU, Jiang-Ning HU (2016) Pharmacokinetics of Daidzein 4'-Phenylsulfonyl Ester and Its Active Metabolite Daidzein Assayed by HPLC Method in Rats After Intravenous Administration and Oral Administration. *Gavin J Food Nutrit Sci* 2016: 1-5.

Received: 26 February, 2016; **Accepted:** 15 March, 2016; **Published:** 29 March, 2016

Abstract

To determine the pharmacokinetics of Daidzein 4'-Phenylsulfonyl Ester (DZPE) in rats, a validated HPLC method has been developed for the simultaneous determination of DZPE and its metabolite Daidzein (DZ) in rat plasma. DZPE and DZ were determined by reversed-phase HPLC system (column: Diamonsil C18, 5 μ m silica, 200 \times 4.6 mm i.d.; eluent: methanol and 0.1% formic acid aqueous solution, 1:1, v/v; detection wavelength: 262 nm) with genistein as the internal standard. The calibration curves were linear over the ranges of 19.8-19800 ng/ml for DZPE and 10.26-8208 ng/ml for DZ. The lower limits of detection were 9.64 ng/ml and 5.42 ng/ml for DZPE and DZ, respectively. The RSDs of intra-days and inter-days tests were less than 8.2% and the average recovery ranged from 86.6% to 94.4% for DZPE and 72.4% to 82.0% for DZ. Subsequently, the method was applied to the pharmacokinetic study of DZPE and DZ in rat's plasma. About 30.6% of DZPE was converted into DZ after oral administration of DZPE in rats, while only 11.2% of DZPE was converted into DZ after intravenous administration of DZPE within 24 h. The absolute bioavailability of total DZPE and DZ was 31.6%. The method was demonstrated to be feasible for pharmacokinetic studies of DZPE in rats.

Keywords

Daidzein; Daidzein 4'-Phenylsulfonyl ester; Pharmacokinetics; RP-HPLC

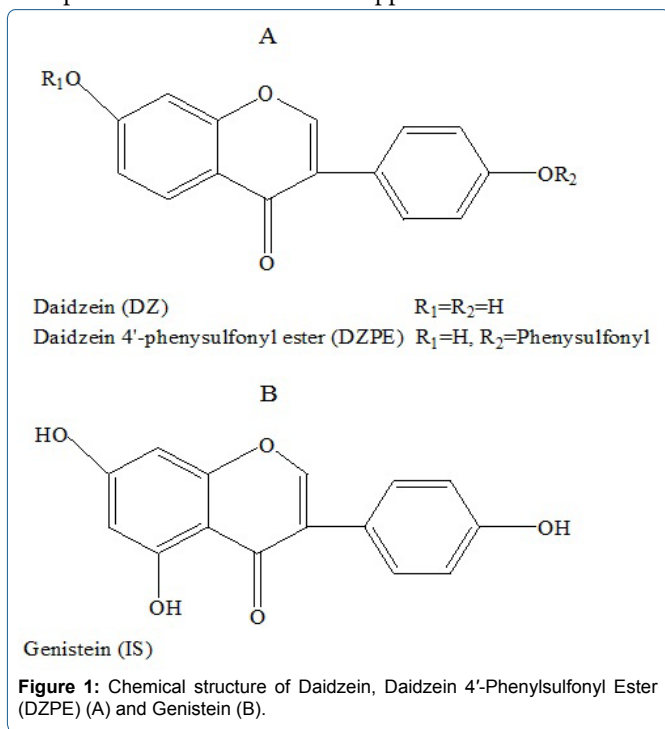
Introduction

As a major isoflavone component of soybeans and *Glycine max* (L.) Merr, the oestrogen-like compound daidzein is widely consumed by humans from the sources of soy and soy-based food products. Many pharmaceutical researches showed that daidzein exhibited various health-benefit activities including inhibition of the growth of breast and prostate cancers [1,2], promotion of the proliferation of osteoporosis cells [3,4] prevention of diabetes onset [5] and alleviation of menopausal

symptom [6-8]. Apart from these beneficial effects on human health, daidzein has also been approved by US Food and Drugs Administration as a good candidate in treating cardiovascular diseases in virtue of the advantages of low cost and abundance as well as its clinical safety [9]. The mechanism of anti-cardiovascular disease of daidzein is probably due to reducing the Low-Density Lipoprotein (LDL) cholesterol, inducing nitric oxide production, and improving vascular reactivity [10].

Regardless of these health benefits, the oral administration of daidzein has recently been reported to encounter a major therapeutic problem due to its poor oral bioavailability which limits its curative effect [11,12]. Qiu et al., reported that the absolute bioavailability of daidzein in rats after administration

of daidzein solution was only 6.1% in the form of total daidzein (free plus conjugated daidzein) [13]. The reasons caused poor bioavailability of daidzein could be attributed to its low solubility, its low partition coefficient of oil/water, and especially its strong metabolism that occurs in the intestine and liver [14]. In order to increase the oral administration of daidzein, which would be expected to increase its tract or cellular absorption, several research groups have already reported to modify the molecular structure of daidzein to create its various derivatives [15-18]. Among them, esterification is usually considered as a better route to obtain its derivatives with better bioavailability. It has been reported that acylation of cholestane could increase the antitumor potency [19]. Acylated saponin in low concentration also activated the metabolism of endothelial cells, which enhanced the permeability of the blood vessel walls for better adsorption of the saponin into tissues [20]. Our research group has also synthesized a sulfonate analogue of daidzein (Figure 1) in previous report, which is expected to enhance its bioavailability [21]. In the present study, we continue to develop a validated HPLC method prior to evaluation of the pharmacokinetics of Daidzein 4'-Phenylsulfonyl Ester (DZPE) and its metabolite Daidzein (DZ) in rats after intravenous administration and oral administration of DZPE. With the results, we may provide an experimental basis for clinical application.



Materials and Methods

Chemical and reagents

Daidzein (DZ) and Genistein (Internal Standard, IS) with purity more than 98% was obtained from Shanxi Huike Plant Company (Shanxi, China). Daidzein 4'-Phenylsulfonyl Ester (DZPE) was synthesized and purified in our lab. HPLC grade

acetonitrile and methanol (CH_3OH) were purchased from Merck Company (Darmstadt, Germany). Dimethylsulfoxide (DMSO), formic acid and other reagents were of reagent grade. Distilled water was used for the preparation of all solutions.

Animals and drug administration

Wistar rats, 8-week old and weighing 200-250 g, were purchased from the Animal Center of Nanchang University (Nanchang, China). Animals had free access to water *ad libitum* until 12 h prior to being used in the experiments. All animal experiments complied with the requirements of the National Act on the Use of Experimental Animals (China). Fifty rats were divided into two groups treated by intravenous administration and oral administration of DZPE, respectively. The oral dose or intravenous dose of DZPE were first dissolved in DMSO and then diluted to the final concentration with PEG-400 (35 mg/kg). Plasma samples were collected at 0.08, 0.17, 0.33, 0.67, 1, 2, 4, 6, 8, 12, 24 h. Then, each blood sample was centrifuged at $14,000 \times g$ for 10 min. The plasma samples were stored at $-70^\circ C$ until analysis by HPLC.

Stock solution and sample preparation

The stock solution of each analyte (DZ and DZPE) was prepared by dissolving appropriate concentration of the analyte in MeOH to achieve a concentration of 205.2 $\mu g/ml$ and 258 $\mu g/ml$, respectively. The stock solution of IS (genistein) was prepared in CH_3OH and diluted with CH_3OH to result in a final concentration of 92 $\mu g/ml$. As for preparation of plasma sample, IS (10 μl) and ethyl acetate (extraction solvent, 1 ml) were added to plasma samples (50 μl), and then vortexed vigorously for 3 min, followed by centrifuging at $14,000 \times g$ for 10 min. The supernatant was transferred to glass tube and evaporated to dryness under a gentle stream of nitrogen at $40^\circ C$. The residue was reconstituted with 200 μl of CH_3OH .

HPLC analytic method

The separation of each sample was performed by Agilent HPLC series 1100 (Agilent Technologies, Little Falls, DE, USA) equipped with quaternary pump, vacuum degasser, autosampler and UV detector. The HPLC system was connected to an Agilent Chemstation Software. Diamonsil C18 column (200×4.6 mm, 5 μm) maintained at $25^\circ C$ was used for a separation. The mobile phase used was CH_3OH : 0.1% formic acid aqatic solution (1:1, v/v). The flow rate was set at 0.5 ml/min and UV-V is detection was performed at 262 nm.

HPLC Method Validation

Linearity, the Lower Limit of Detection (LLOD) and Lower Limit of Quantitation (LLOQ)

In order to construct the calibration curve, a series of each analyte (DZ and DZPE) standard solutions for plasma were prepared by spiking blank rat plasma with appropriate stock solution of each analyte and IS stock solutions (20 μl). The final concentrations of each analyte were 10.26, 20.52, 61.56, 205.2,

820.8, 3283.2, 8208 ng/ml. The samples were then treated by the above extraction procedure and analyzed. Standard curves were plotted from tested compound/IS peak area ratios vs tested compound concentrations. Meanwhile, analytic method validation including the Lower Limit of Detection (LLOD) and Lower Limit of Quantitation (LLOQ) were determined. The LLOD and LLOQ under the present chromatographic condition were defined as a signal to noise (S/N) ratio of 3 and 10 respectively.

Accuracy and precision

To study the reliability and suitability of the developed method, plasma samples were spiked with three concentration of DZ (20, 80, 450 ng/ml) and DZPE (40, 100, 1000 ng/ml). The intra-day accuracy and precision values were determined on one day, while the inter-day accuracy and precision were determined for 3 consecutive days. Six replicates were analyzed in each of three analytical runs.

Recovery

The recoveries of DZ and DZPE from rat plasma were evaluated at three concentrations DZ (20, 80, 450 ng/ml) and DZPE (40, 100, 1000 ng/ml) covering the linear range of the standard curve. Each analyte was subsequently analyzed, and quantified from the corresponding calibration curve. The recovery test is determined as: $\text{Recovery (\%)} = (\text{Amount}_{\text{determined}} - \text{Amount}_{\text{spiked}}) / \text{Amount}_{\text{spiked}} \times 100\%$. Where $\text{Amount}_{\text{determined}}$ is the determined total amount from plasma sample and $\text{Amount}_{\text{spiked}}$ is the spiked amount of each standard.

Stability

Short-term and long-term stability of DZ and DZPE in plasma samples were assessed at three concentrations (20, 800 and 3200 ng/ml) for DZ and (40, 1000 and 10000 ng/ml) for DZPE. The short-term stability was studied by analysis of the samples being left at 20°C for 24 h and the long-term stability by analysis of the samples after storage at -70°C for 30 days. The freeze-thaw stability was determined after three freeze and thaw cycles. In each cycle, the samples were stored at -20°C for 24 h and thawed unassisted at room temperature. When completely thawed, the sample was refrozen within 24 h. The cycle was repeated two times and then analyzed.

Pharmacokinetic study

Pharmacokinetic analysis was carried out using DAS 2.1.1 pharmacokinetic calculation software (Chinese Society of Mathematical Pharmacology, China). Area Under the Curve (AUC), maximum plasma concentration (C_{max}) and the time needed to reach the maximum plasma concentration (T_{max}) were determined by the program. The elimination rate constant (K_{el}) was obtained from the terminals slope using regression analysis, and the half-life ($t_{1/2}$) of the drug was calculated by a relationship of $0.693/K_{\text{el}}$.

Results and Discussion

Analytic method development

Typical chromatograms displaying the peaks observed in plasma sample for DZ or DZPE spiked with IS are shown in figure 2. The retention times of DZ, DZPE and IS were 12.37, 13.44, 15.25 min, respectively. Good separations between IS and DZ or DZPE were achieved under the chromatographic conditions used. No interfering peaks from endogenous substances were observed. Standard curves were plotted from tested compound/IS peak area ratios vs tested compound concentrations in the ranges of 10.26 - 8208 ng/ml for DZ and 19.8-19800 ng/ml for DZPE, respectively. The linear equation of DZ obtained was $Y = 6 \times 10^{-5} X + 0.013$ ($r^2 = 0.9999$), while the linear equation of DZPE obtained was $Y = 0.002X + 0.0317$ ($r^2 = 0.9998$). The standard curves exhibited excellent linearity and correlation coefficient. The limits of the lowest concentration on the calibration curve can be measured with acceptable RSD < 10%. The LLODs for DZ and DZPE were 5.42 ng/ml and 9.64 ng/ml, while the LLOQs for DZ and DZPE were 10.26 ng/ml and 19.8 ng/ml in rat plasma, respectively.

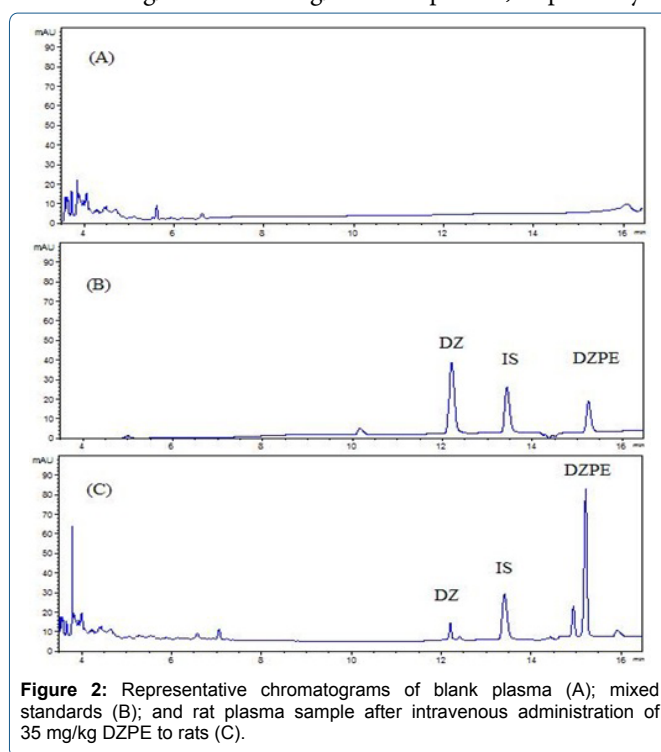


Figure 2: Representative chromatograms of blank plasma (A); mixed standards (B); and rat plasma sample after intravenous administration of 35 mg/kg DZPE to rats (C).

Method Validation

Precision and recovery

The accuracy and precision are the most important criteria for judging the performance of an analytical method. The accuracy of the analytical method is estimated as the percentage difference between the mean values generated by the method and the known concentrations (relative error, %e_r). The precision is expressed as the percentage Relative Standard Deviation (RSD%). Table 1 gave the overview of the intra-day

RSD, inter-day RSD and mean RE% of the precision and accuracy for low, medium and high quality controls. The intra- and inter-day accuracies (RE%) of DZ and DZPE were -1.8-7.5%, 0.2- 7.7%, -12.5 - 5.0% and -6.8 - 10.5%, respectively. The intra- and inter-day precisions (RSD%) of DZ and DZPE were not more than 6.1, 6.7, 7.6, and 8.2%, respectively. The extraction recoveries of DZ from rat plasma were $72.4 \pm 14.0\%$, $82.0 \pm 6.3\%$, and $81.3 \pm 4.4\%$ at concentrations of 20, 80, 450 ng/ml, while the recoveries of DZPE were $86.6 \pm 0.9\%$, $90.7 \pm 0.7\%$, and $94.4 \pm 3.9\%$ at concentrations of 40, 100, 1000 ng/ml, respectively. All the results of the tested samples met the requirements of biological analysis.

35 mg/kg DZPE in rats are illustrated in figure 3. The main pharmacokinetic parameters of DZPE and DZ in rats are summarized in table 3. Plasma level of DZ steadily increased after oral administration of DZPE within 1 h. Most DZ was eliminated within 24 h after administration. The $AUC_{(0-1)}$ and $AUC_{(0-\infty)}$ of DZ measured after oral administration of DZPE were estimated to be 1102 ± 445 and 1255 ± 537 ng h/ml, respectively. DZ was found in plasma after either intravenous or oral administration and 30.6% of DZPE was converted into DZ after oral administration, while only 11.2% of DZPE was converted into DZ after intravenous administration within 24 h. This is mainly due to the effect of liver esterases on DZPE

	Intra-day procession (n=6)			Inter-day procession (n=6)		
DZPE						
Spiked concentration (ng/ml)	40	100	1000	40	100	1000
Measured concentration (ng/ml)	35.46 ± 1.24	103.31 ± 7.85	1050.27 ± 32.5	37.26 ± 2.75	110.57 ± 9.1	1034.83 ± 82.7
RE (%)	-12.5	3.0	5.0	-6.8	10.5	3.4
RSD (%)	3.5	7.6	3.1	7.4	8.2	8.0
DZ						
Spiked concentration (ng/ml)	20	80	450	20	80	450
Measured concentration (ng/ml)	20.08 ± 0.85	86.24 ± 3.26	442.1 ± 26.96	20.04 ± 1.2	86.12 ± 4.13	442.25 ± 29.63
RE (%)	0	7.5	-1.8	0.2	7.6	7.7
RSD (%)	4.1	3.8	6.1	6.0	4.8	6.7

Table 1: Precision and accuracy of the LC method for determining DZPE and DZ in rat plasma (mean \pm SD).

Stability

Table 2 showed that plasma samples containing DZ and DZPE were stable at 20°C for 24 h and at -70°C for 30 days. Meanwhile, the rat plasma samples were stable after three freeze-thaw cycles. No appreciable loss of DZ and DZPE was detected within the experimental conditions.

Spiked concentration (ng/ml)	DZPE			DZ		
	40	1000	10000	20	800	3200
Storage stability (-70°C, 30 d)						
Measured concentration (ng/ml)	41.8	1053	9600	20.68	771.2	3443
RE (%)	4.5	5.3	-4.0	3.4	-3.6	7.6
Freeze-thaw stability (3 cycles)						
Measured concentration (ng/ml)	38.36	1009	11060	18.94	760	3347
RE (%)	-4.1	0.9	-10.6	-5.3	-5.0	4.6
Processed stability (20°C, 24 h)						
Measured concentration (ng/ml)	44.12	1102	9110	18.94	823.2	3324
RE (%)	10.3	10.2	-8.9	-5.3	2.9	3.9

Table 2: Stability of DZPE and DZ in rat plasma at different conditions (n=6).

Pharmacokinetic study after intravenous and oral administration of DZPE

The profiles of total DZ and DZPE plotted against time after oral administration and intravenous administration of

as well as plasma esterases on DZPE following oral administration [22]. The absolute bioavailability of the sum of DZPE and DZ was 31.6%, which indicated that the ester-containing compound DZPE improved the absorption compared to the absolute bioavailability of DZ (6.1%) as reported from the previous study [13].

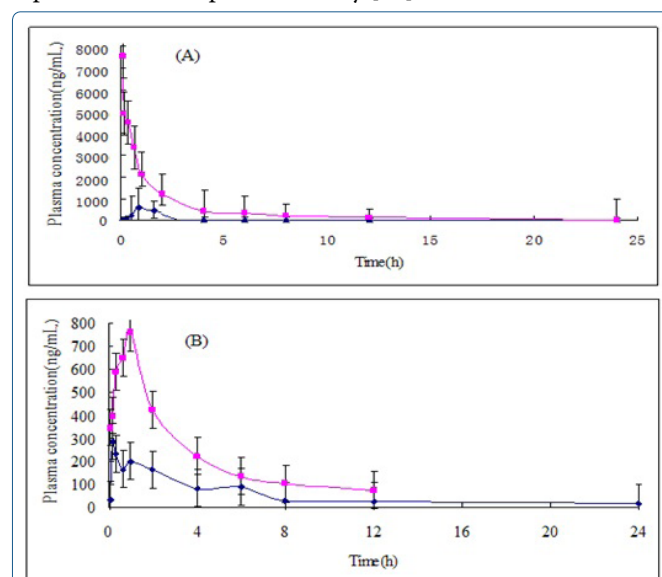


Figure 3: The mean plasma concentration-time curves of DZPE (■) and DZ (♦) after intravenous administration (A) and oral administration (B) of 35 mg/kg DZPE to rats (n=5).

In summary, we have developed a simple HPLC method with necessary precision, sensitivity, and accuracy to allow the simultaneous determination of DZ and DZPE in rat plasma.

Parameters	Oral		Intravenous	
	DZPE	DZ	DZPE	DZ
T _{max} (h)	0.8 ± 0.30	0.37 ± 0.36	–	0.7 ± 0.16
C _{max} (ng/ml)	930 ± 351	324 ± 72	–	716 ± 129
t _{1/2} (h)	3.26 ± 1.72	5.44 ± 4.11	3.10 ± 0.73	6.94 ± 7.13
MRT _{0-t} (h)	2.96 ± 0.79	4.27 ± 2.15	2.94 ± 1.01	4.29 ± 2.85
AUC _{0-t} (ng.h/ml)	2490 ± 570	1102 ± 445	10086 ± 804	1267 ± 432
AUC _{0-∞} (ng.h/ml)	2953 ± 658	1255 ± 537	10119 ± 829	1415 ± 596
Absolute bioavailability (%)	31.6			

Table 3: The mean non-compartmental pharmacokinetic parameters of DZPE and DZ in rats after single oral and intravenous administration of 50 mg/kg (n=5).

Meanwhile, the validated HPLC method has been successfully applied to study the pharmacokinetics in rats following intravenous and oral administration of DZPE at doses of 35 mg/kg. The metabolism of DZPE to DZ after oral administration was greater than that after intravenous administration in rats, and the relative bioavailability of the sum of DZPE and DZ was 31.6%.

References

- Jin S, Zhang QY, Kang XM, Wang JX, Zhao WH (2010) Daidzein induces MCF-7 breast cancer cell apoptosis via the mitochondrial pathway. *Ann Oncol* 21: 263-268.
- Boersma BJ, Barnes S, Kirk M, Wang CC, Smith M, et al. (2001) Soy isoflavonoids and cancer-metabolism at the target site. *Mutat Res* 480-481: 121-127.
- Coxam V (2008) Phyto-oestrogens and bone health. *Proceedings of the Nutrition Society* 67: 184-195.
- Barnes S (2003) Phyto-oestrogens and osteoporosis: what is a safe dose? *British Journal of Nutrition* 89: 101-108.
- Choi MS, Jung UJ, Yeo J, Lee MK (2008) Genistein and daidzein prevent diabetes onset by elevating insulin level and altering hepatic gluconeogenic and lipogenic enzyme activities in Non-Obese Diabetic (NOD) mice. *Diabetes Metab Res Rev* 24: 74-81.
- Ishiwata N, Melby MK, Mizuno S, Watanabe S (2009) New equol supplement for relieving menopausal symptoms: randomized, placebo-controlled trial of Japanese women. *Menopause* 16: 141-148.
- Murkies AL, Lombard C, Strauss BJ, Wilcox G, Burger HG, et al. (2008) Dietary flour supplementation decreases post-menopausal hot flushes: effect of soy and wheat. *Maturitas* 61: 27-33.
- Baber RJ, Templeman C, Morton T, Kelly GE, West L (1999) Randomized placebo-controlled trial of an isoflavone supplement and menopausal symptoms in women. *Climacteric* 2: 85-92.
- Rimbach G, Boesch-Saadatmandi C, Frank J, Fuchs D, Wenzel U (2008) Dietary isoflavones in the prevention of cardiovascular disease- a molecular perspective. *Food Chem Toxicol* 46: 1308-1319.
- Gao Y, Gu WW, Chen LL, Xu ZH, Li YP (2008) The role of daidzein-loaded sterically stabilized solid lipid nanoparticles in therapy for cardio-cerebrovascular diseases. *Biomaterials* 29: 4129-4136.
- Allred CD, Twaddle NC, Allred KF, Goeppinger TS, Churchwell MI, et al. (2005) Soy processing affects metabolism and disposition of dietary isoflavones in ovariectomized BALB/c mice. *J Agric Food Chem* 53: 8542-8550.
- Zheng Y, Lee SO, Verbruggen MA, Murphy PA, Hendrich S (2004) The apparent absorptions of isoflavone glucosides and aglucons are similar in women and are increased by rapid gut transit time and low fecal isoflavone degradation. *J Nutr* 134: 2534-2539.
- Qiu F, Chen XY, Song B, Zhong DF, Liu CX (2005) Influence of dosage forms on pharmacokinetics of daidzein and its main metabolite daidzein-7-O-glucuronide in rats. *Acta Pharmacol Sin* 26: 1145-1152.
- Kulling SE, Honig DM, Metzler M (2001) Oxidative metabolism of the soy isoflavones daidzein and genistein *in vitro* and *in vivo*. *J Agric Food Chem* 49: 3024-3033.
- Fairley B, Botting NP, Cassidy A (2003) The synthesis of daidzein sulfates. *Tetrahedron* 59: 5407-5410.
- Green JM, Alvero AB, Kohen F, Mor G (2009) 7-(O)-carboxymethyl daidzein conjugated to N-t-Boc-hexylendimine: a novel compound capable of inducing cell death in epithelial ovarian cancer stem cells. *Cancer Biol Ther* 8: 1747-1753.
- Seeger M, González M, Cámara B, Muñoz L, Ponce E, et al. (2003) Biotransformation of natural and synthetic isoflavonoids by two recombinant microbial enzymes. *Appl Environ Microbiol* 69: 5045-5050.
- Rusin A, Krawczyk Z, Gryniewicz G, Gogler A, Zawisza-Puchalka J (2010) Synthetic derivatives of genistein, their properties and possible applications. *Acta Biochim Pol* 57: 23-34.
- Mimaki Y, Kuroda M, Kameyama A, Sashida Y (1997) Cheolestane glycosides with potent cytostatic activities on various tumor cells from *Ornithogalum saundersiae* bulbs. *Bioorganic & Medicinal Chemistry Letter* 7: 633-636.
- Melzig MF, Bader G, Loose R (2001) Investigation of the mechanism of membrane activity of selected triterpenoid saponins. *Planta Med* 1: 43-48.
- Peng Y, Deng ZY, Lang SJ, Xiong DM (2007) 3-(4-Hydroxyphenyl)-7-(phenylsulfonyl)-4H-chromen-4-one. *Acta Cryst* 63: 4787.
- Los LE, Welsh DA, Herold EG, Bagdon WJ, Zacchei AG (1996) Gender differences in toxicokinetics, liver metabolism, and plasma esterase activity: observations from a chronic (27-week) toxicity study of enalapril/diltiazem combinations in rats. *Drug Metab Dispos* 24: 28-33.