

**PM METHOD DEVELOPMENT
STUDY SUMMARY**

P. mirifica Fluid Extracts – Method Development Results Summary

A. Standards Purity and UV spectra

Purity: As area % at 255nm and 260nm

	<u>source</u>	<u>255nm</u>	<u>260nm</u>
puerarin	Triarco	87.2	85.6
daidzin	indofine	97.2	97.3
genistin	indofine	>99	>99
daidzein	indofine	>99	>99
genistein	indofine	>99	>99

Standards data in files PMIRIF and PMIRIFD

HP spectral purity analysis on individual peaks all pass.

B. Instrument Limit Studies; 7 replicate injections at ca. 0.2 ug/mL and ca. 1.0 ug/mL

Instrument Detection Limits: (t)(std. dev.), where $t=2.447$ at 95% confidence, $n-1=6$

	<u>ca. 0.2 ug/mL</u>	<u>ca. 1.0 ug/mL</u>
puerarin	0.40ng	0.14ng
daidzin	0.20ng	0.19ng
daidzein	0.07ng	0.24ng
genistin	0.11ng	0.23ng
genistein	0.09ng	0.21ng

Instrument Quantification Limits: (10)(std. dev.)

	<u>ca. 0.2 ug/mL</u>	<u>ca. 1.0 ug/mL</u>
puerarin	1.64ng	0.55ng
daidzin	0.84ng	0.78ng
daidzein	0.30ng	0.98ng
genistin	0.44ng	0.94ng
genistein	0.39ng	0.86ng

C. Linearity

Two 3-point calibrations over a range of 100 were examined for linearity during the course of accuracy/precision studies and actual sample analysis.

Fluid Extract Sample Analysis: 0.2, 2.0, and 20 ug/mL – All target analytes gave a linear response over this range with all correlation coefficients > 0.9999. Although response was linear, the low point calibration gave errors of over 10% for genistin

(11.4%) and genistein (12.6%). Therefore, further work was carried out with a low point calibration standard of 0.5 ug/mL.

Precision/accuracy Studies: 0.5, 5.0, and 50 ug/mL - All target analytes gave a linear response over this range with all correlation coefficients > 0.9999. The largest error in the low point calibration was for puerarin (3.3%).

D. Accuracy/Precision

Six replicates of a partially fortified fluid extract were analyzed according to the developed method. RSD values and spike recovery were determined. The actual concentrations measured ranged from a low of 0.16 mg/g (genistin) to a high of 0.86 mg/g (puerarin). The fluid extract was fortified as follows:

puerarin	0 ug
daidzin	202ug
daidzein	218ug
genistin	0ug
genistein	418ug

Results:	<u>Analyte</u>	<u>RSD (%)</u>	<u>Recovery (%)</u>
	puerarin	1.05	NA
	daidzin	0.90	93.0
	daidzein	1.95	98.8
	genistin	1.55	NA
	genistein	3.03	92.0

During the course of an actual analysis of a sample group, a duplicate and spiked sample were run with the following results: (spike level 0.2 mg each analyte)

<u>Analyte</u>	<u>RSD (%)</u>	<u>Recovery (%)</u>
puerarin	0.03	99.9
daidzin	0.18	100.0
daidzein	0.93	100.0
genistin	0.44	99.6
genistein	0.17	99.8