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Characterization of Flavonoids and Phytoestrogens in an Extract of Pueraria Mirifica by UHPLC-MS-MS

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1.Introduction

Pueraria mirifica [Leguminosae] is a plant species native to Thailand whose tuberous roots are used medicinally for their anti-ageing properties. P. mirifica is a source of several phytoestrogens and flavonoids such as deoxymiroestrol, puerarin, genistein, and many others. Renewed interest in the herb as well as higher standards of identity and purity demand accurate and precise methods for detecting the various compounds in P. mirifica extracts both qualitatively and quantitatively. [1–3]

UHPLC-MS-MS is an effective strategy to characterize such extracts and accurately measure its key components. A selective and sensitive UHPLC-MS-MS method meeting these requirements was developed.

Fig. 1 Roots of Pueraria Mirifica

2. Methods

A Shimadzu Nexera UHPLC with an LCMS-8040 fast-scanning triple quadrupole mass spectrometer was used for analysis. A Shimpack XR-ODS III column (1.6 µm, 2 × 50 mm) was used with a mobile phase of 0.1% formic acid and acetonitrile. The column temperature was 50°C and the injection volume was 5 µL. ESI or APCI ionization was used either independently, or in a combined dual ionization source (DUIS). Continuous polarity switching was used to enable measurement in both positive and negative mode throughout each run.

LC Column	: XR-ODS III (2 × 50 mm, 1.6 µm)	**Column Temp**	: 50°C
Mobile Phase A	: 0.1% Formic Acid	**Nebulizing Gas**	: 3 L/min
Mobile Phase B	: Acetonitrile	**Drying Gas**	: 15 L/min
Flow Rate	: 0.5 mL/min	**DL Temp**	: 300°C
Probe Voltage	: 4.5 kV (+); 3.5 kV (–)	**Heat Block Temp**	: 200°C

	Ret. Time	Channel	Peak #	Area	Height	Conc (ng/mL)	
Puerarin	4.084	Ch1254nm	17	228007	90540	8250	*
Daidzin	4.427	Ch1254nm	19	55601	26514	2012	**
Genistin	4.847	Ch1254nm	23	16461	8938	335.6	***
Daidzein	5.526	Ch1254nm	28	23838	12704	486	*
Genistein	6.071	Ch1254nm	31	9341	4960	190	***
Deoxymiroestrol (total)	6.463	Ch1254nm	34	11470	4752	234	***

* Calculated by LCMS with external standards
** Calculated by puerarin relative UV response
*** Calculated by dadzein relative UV response

Calculated results for quantitative analysis of selected flavonoids in Pueraria Mirifica

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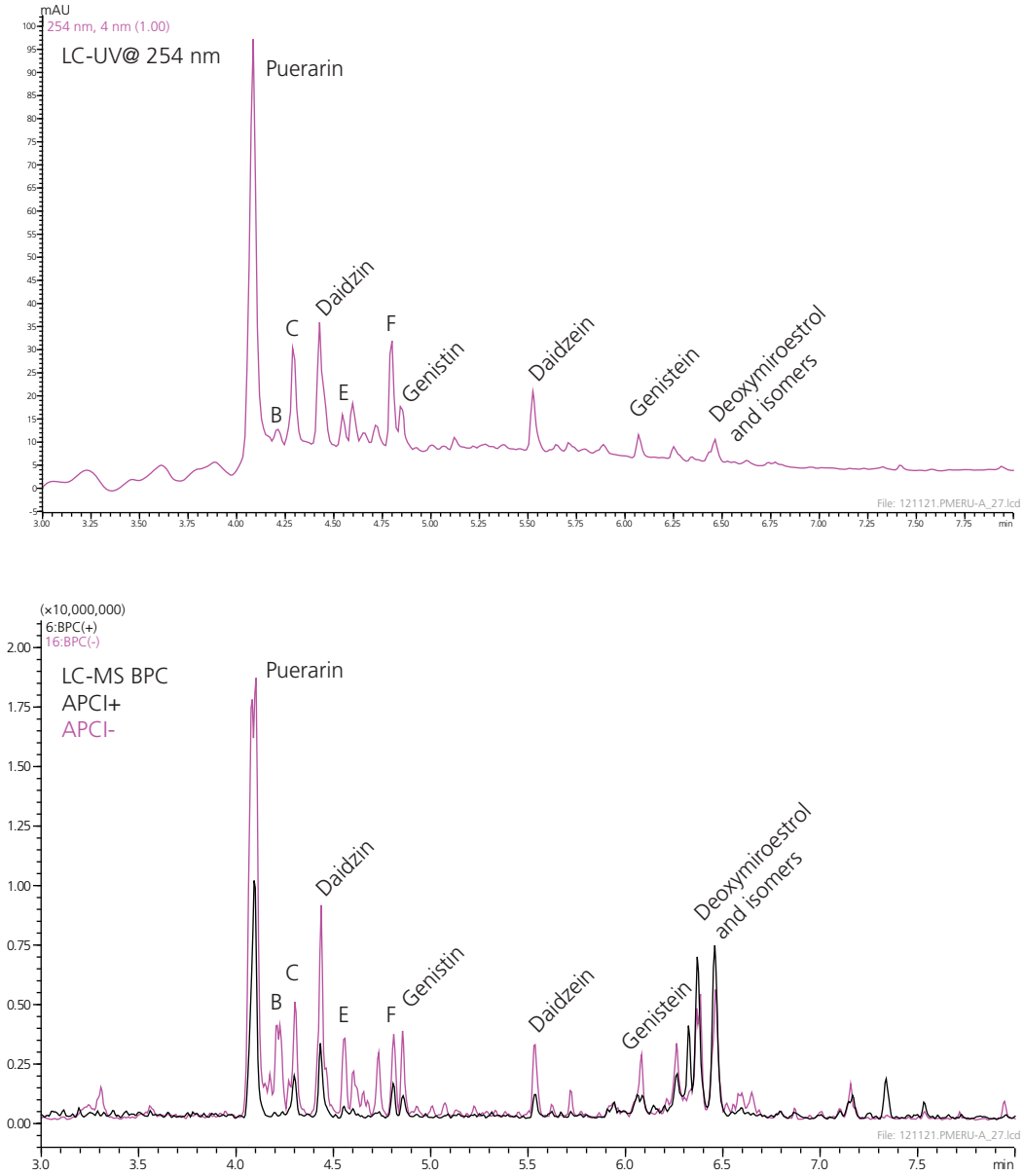

Fig. 2 LC-UV chromatogram (top) and LC-MS base peak chromatogram (bottom) of an injection of the P. mirifica extract.

3. Results and Discussion

MS measurement was carried out in several modes, including full scan, selected ion monitoring, multiple reaction monitoring, and product ion scan modes. Tandem mass spectra were compared with authentic standards or published spectra to propose identifications for each compound. In particular, the tandem mass spectra of O-linked and C-linked flavonoid glycosides were examined and could be distinguished based on the fragmentation patterns of the glycoside ring. In addition, several isomers of deoxymiroestrol were detected and identified based upon their similar fragmentation patterns.

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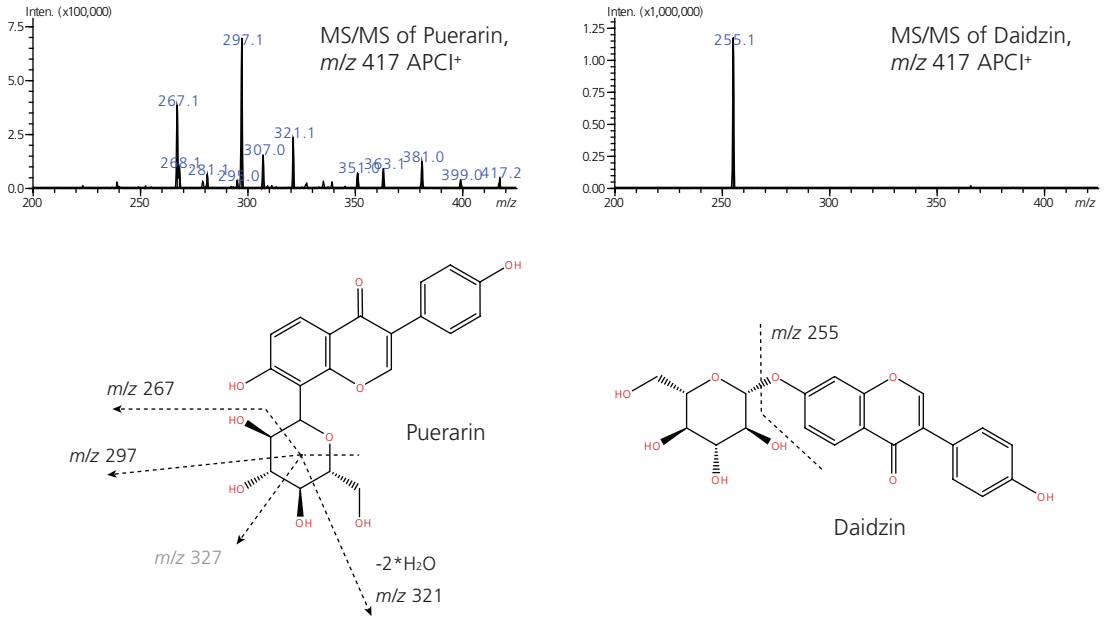

Fig. 3 Tandem mass spectra of puerarin and the peak identified as daidzin in positive mode APCI (Top left and right, respectively). Structure assignment for fragments of puerarin (bottom left) and daidzin (bottom right). The C-linked glycoside puerarin fragments differently than the O-linked glycoside daidzin.

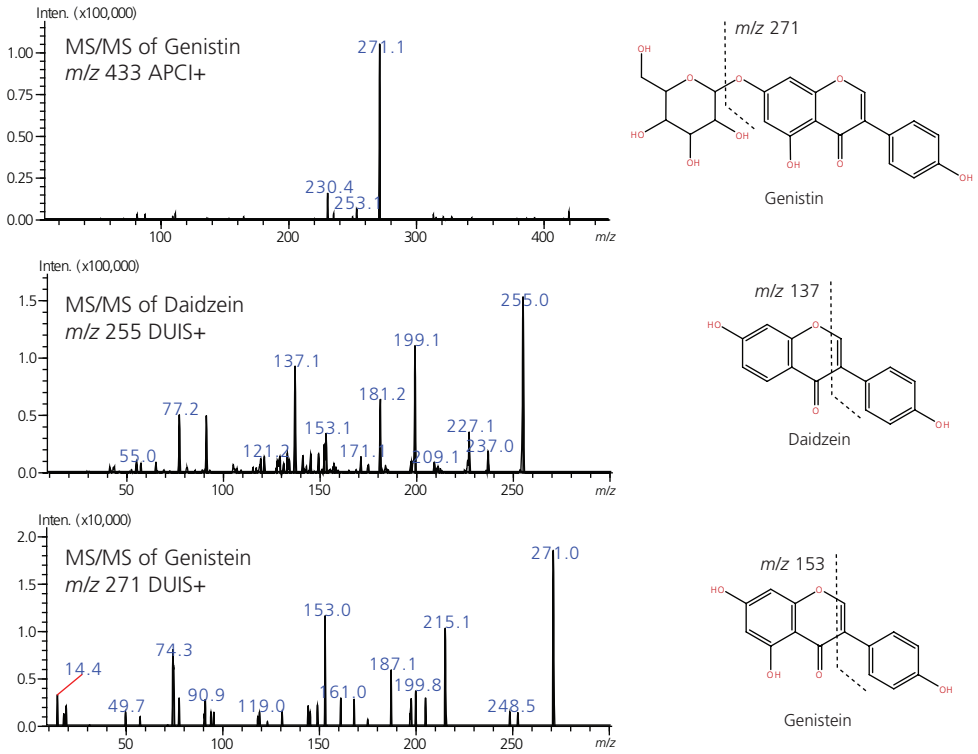

Fig. 4 Tandem mass spectrum and structure assignment of genistin, daidzein, and genistein.

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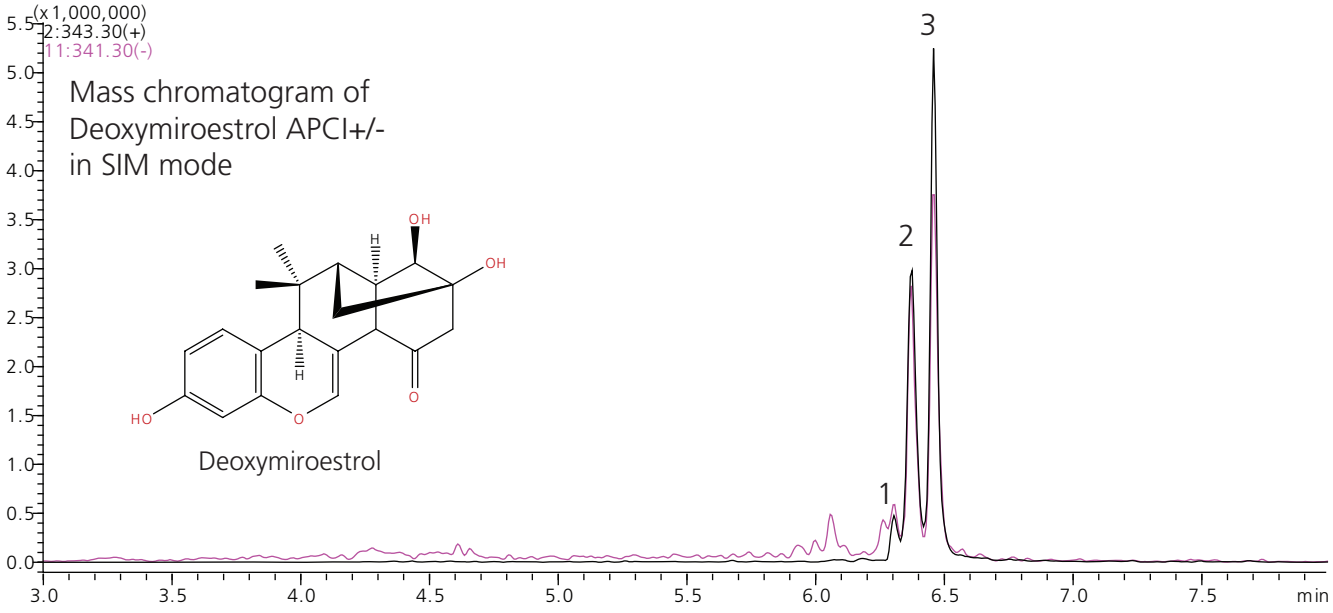

Fig. 5 Mass chromatogram of deoxymiroestrol in APCI with polarity switching. Several isomers of deoxymiroestrol, labeled 1–3, can be clearly distinguished

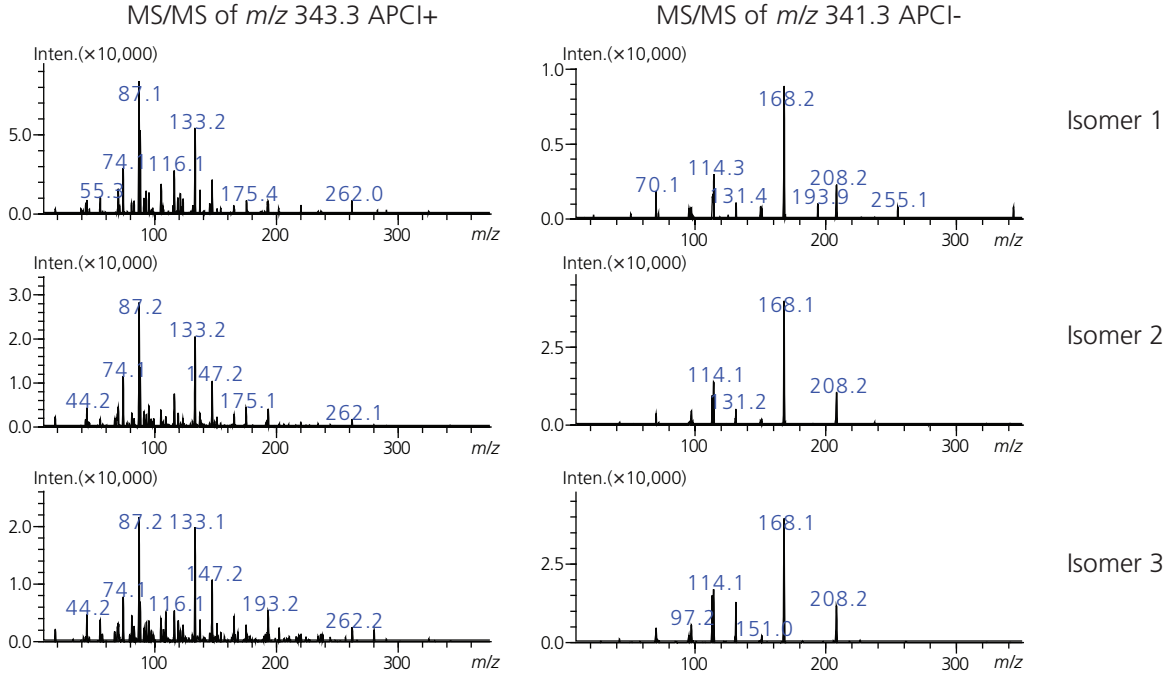

Fig. 6 Tandem mass spectra of three isomers of deoxymiroestrol. The left column are APCI+ spectra and the right column are APCI- spectra. Each row corresponds to one of the isomers of deoxymiroestrol.

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4. Conclusion

UHPLC-MS-MS was used to characterize an extract of P. mirifica, simultaneously revealing several O- and C-linked glycosides as well as several newly detected isomers of deoxymiroestrol. The fast scan speed and rapid polarity switching of the LCMS-8040 triple quadrupole mass spectrometer allowed a full range of data to be acquired for each run with ESI and APCI ionization (DUIS mode), enabling comprehensive characterization of the extract.

5. References

[1] Ching-Che Lin et al. (2005) J. Sep. Sci. 28, 1785–1795
[2] Raymond E. March at al. (2004) Int. J. Mass Spectrom. 232, 171–183
[3] Satoko Shimokawa et al. (2012) Nat. Prod. Res. 27, 371–378

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