

Functional analysis of members of the isoflavone and isoflavanone *O*-methyltransferase enzyme families from the model legume *Medicago truncatula*

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Supplementary Material

Figure S1a-n. HPLC chromatograms of MtIOMT reaction products

Figure S2. Positive ion LC/ESI/MSMS spectra of reaction products from liquiritigenin

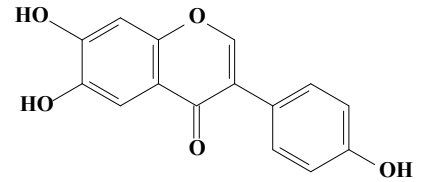
Figure S3. Positive ion LC/ESI/MSMS spectra of reaction products from naringenin

Figure S4. Positive ion LC/ESI/MSMS spectra of reaction products from dihydrodaidzein

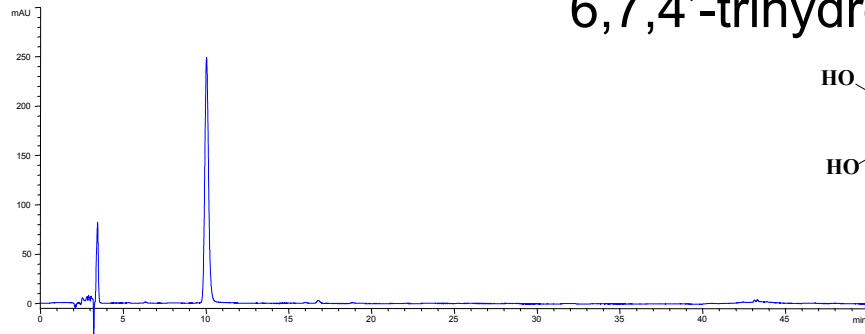
Table S1. Primers used for RT-PCR

Table S2. Quantification of methylated products in reactions with dihydrodaidzein

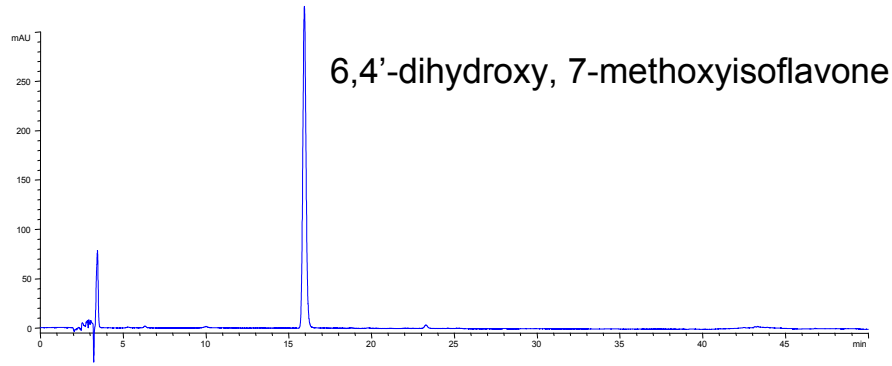
6,7,4'-trihydroxyisoflavone



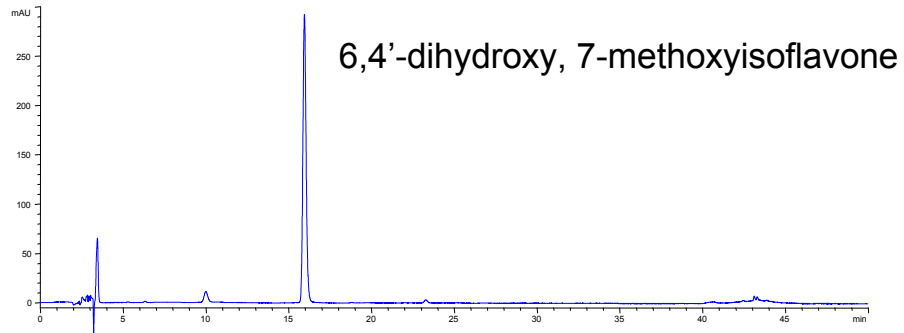
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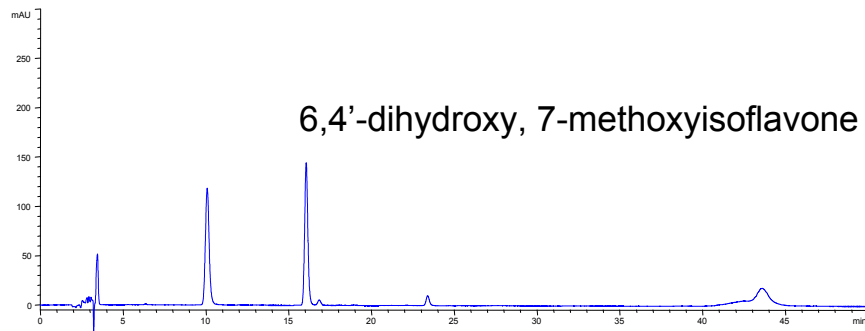
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MtIOMT2



MtIOMT3



MtIOMT4

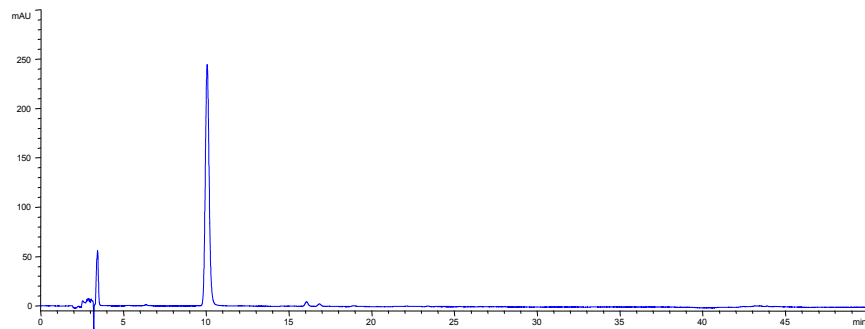
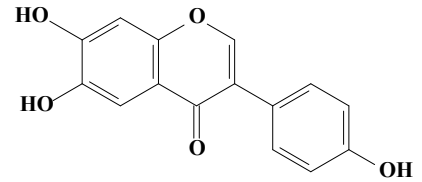
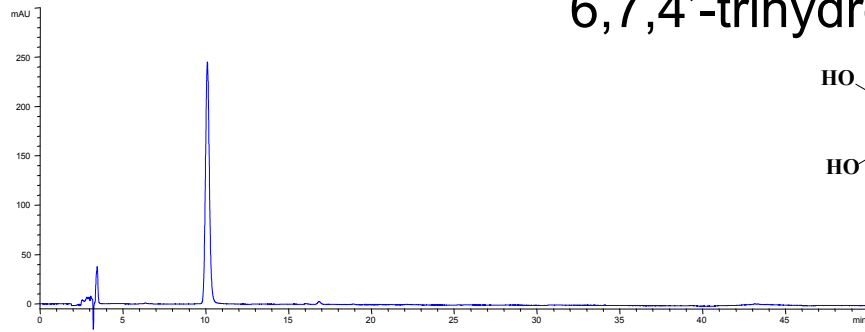


Figure S1(a). HPLC traces of reaction products with 6,7,4'-trihydroxyisoflavone

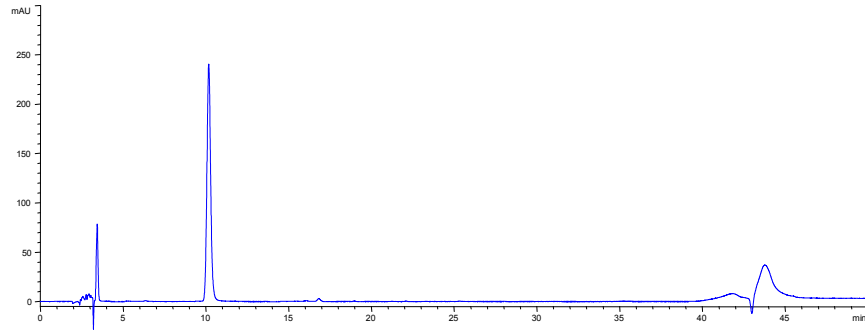
6,7,4'-trihydroxyisoflavone



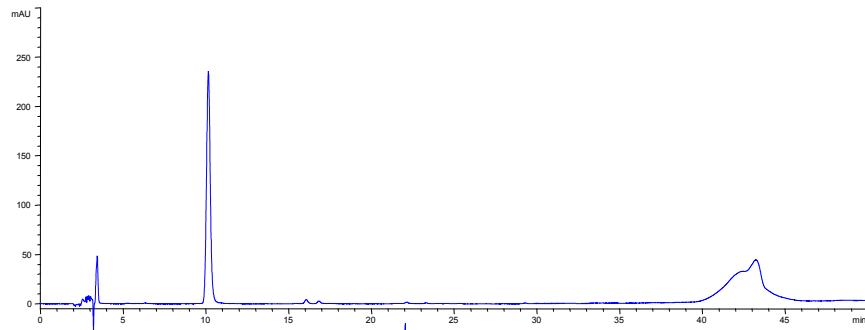
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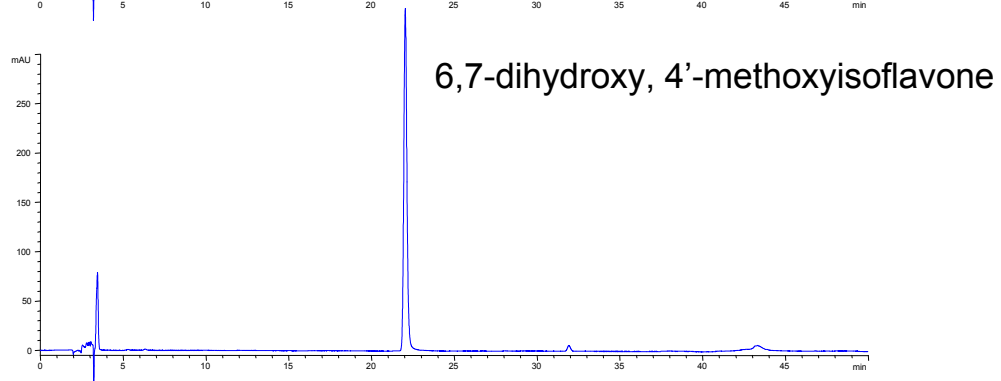
MtIOMT6



MtIOMT7



Standard



Standard

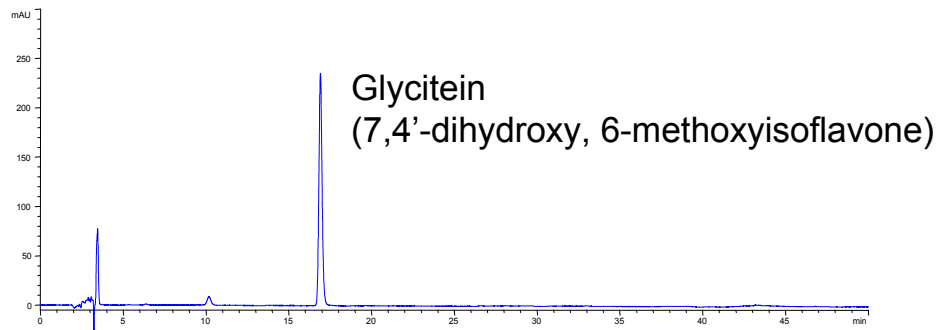
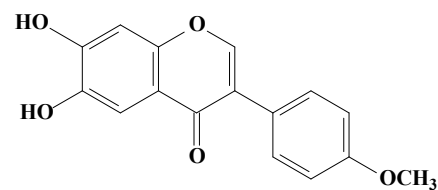
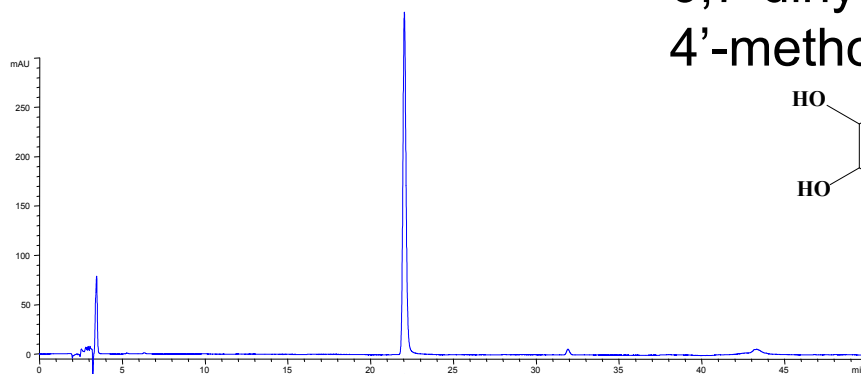


Figure S1(a), cont. HPLC traces of reaction products with 6,7,4'-trihydroxyisoflavone

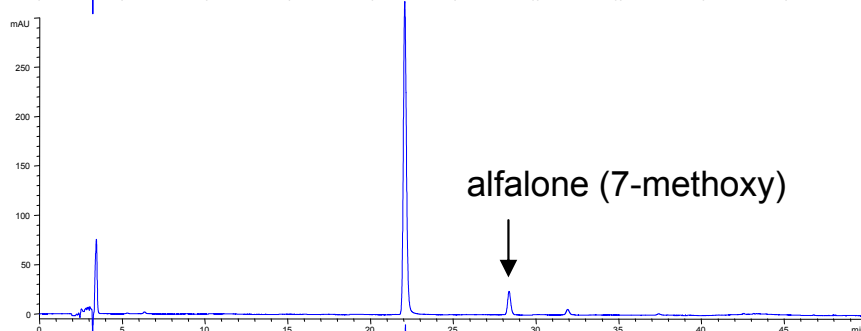
6,7-dihydroxy, 4'-methoxyisoflavone



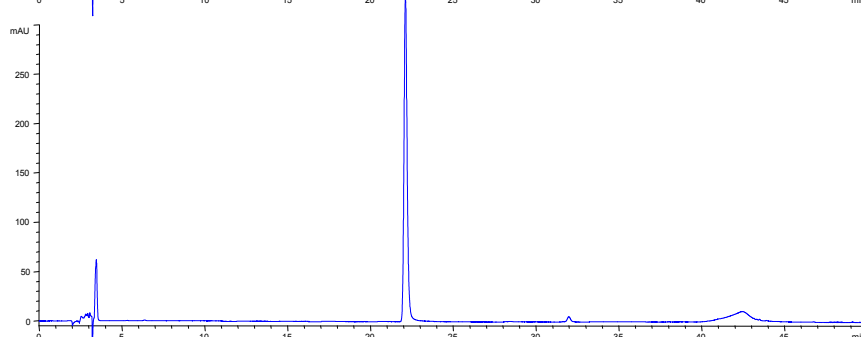
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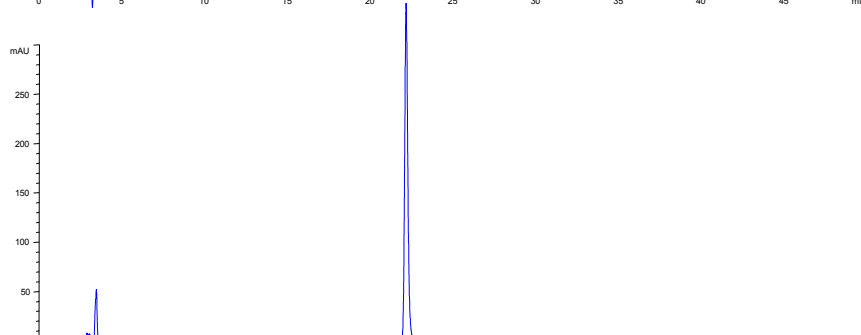
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MtIOMT2



MtIOMT3



MtIOMT4

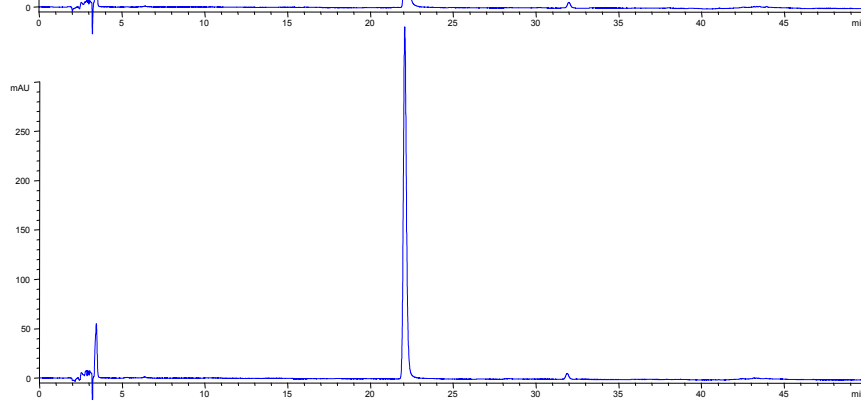
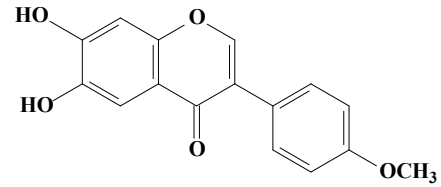
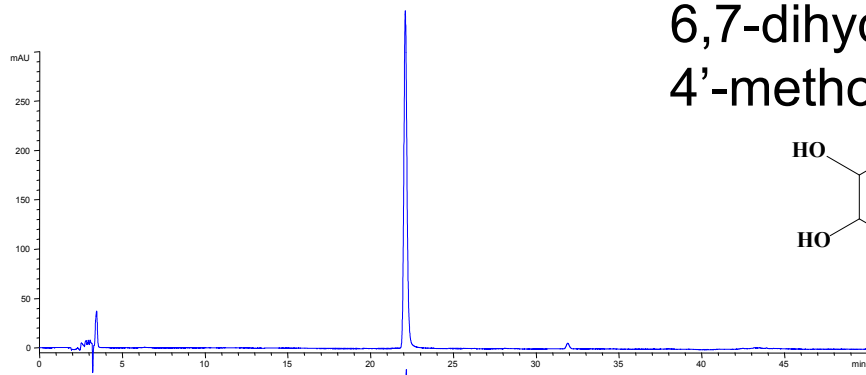


Figure S1(b). HPLC traces of reaction products with 6,7-dihydroxy, 4'-methoxyisoflavone

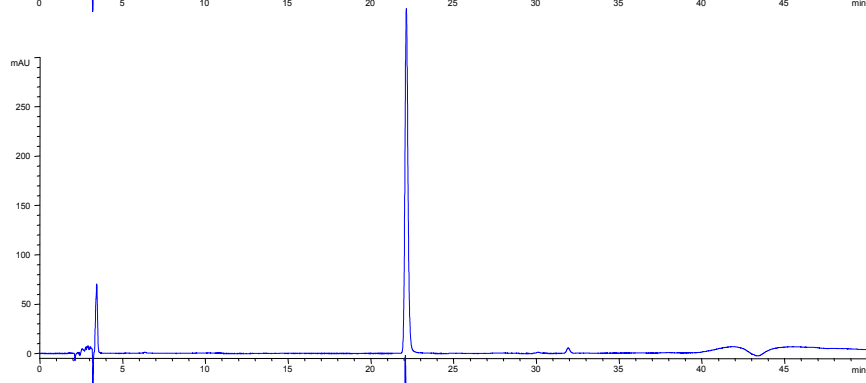
6,7-dihydroxy,
4'-methoxyisoflavone



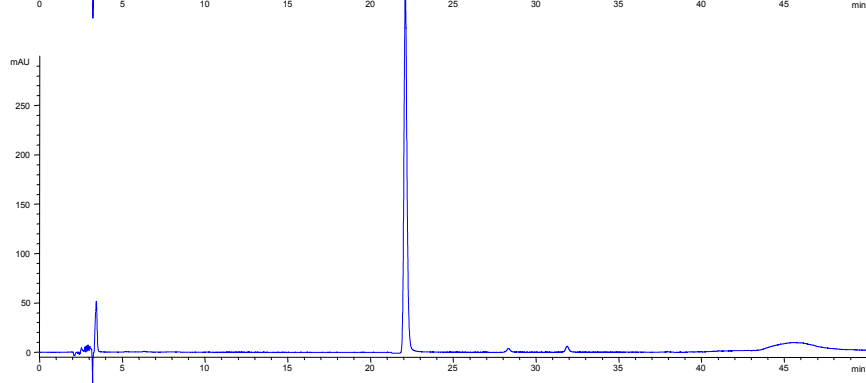
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MtIOMT6

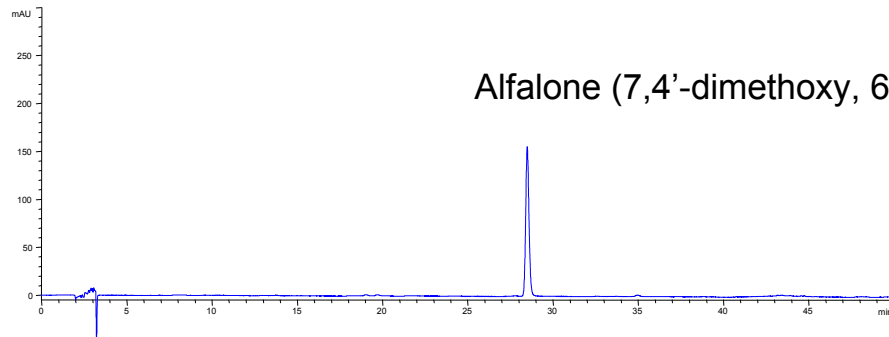


MtIOMT7



Standard

Alfalone (7,4'-dimethoxy, 6-hydroxyisoflavone)



Standard

Afromosin (6,4'-dimethoxy, 7-hydroxyisoflavone)

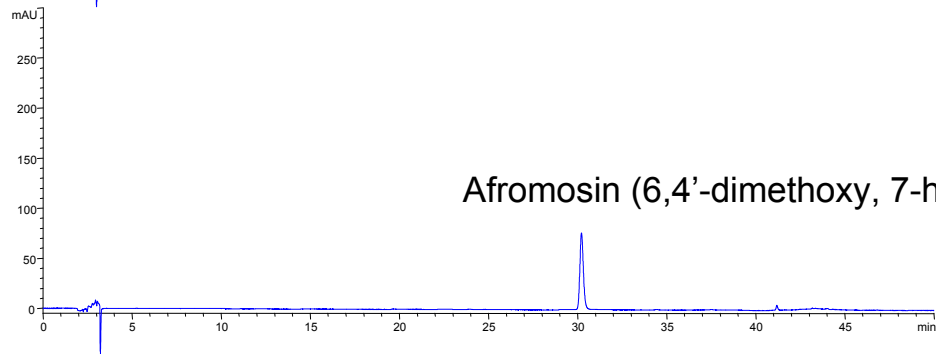
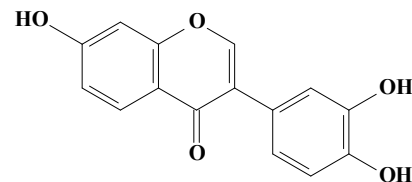
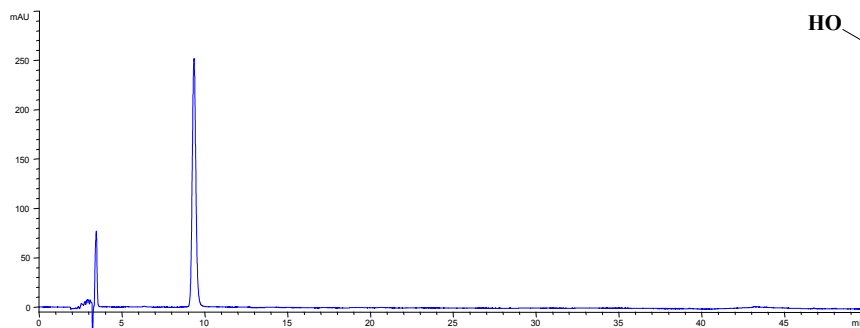


Figure S1(b), cont. HPLC traces of reaction products with 6,7-dihydroxy, 4'-methoxyisoflavone

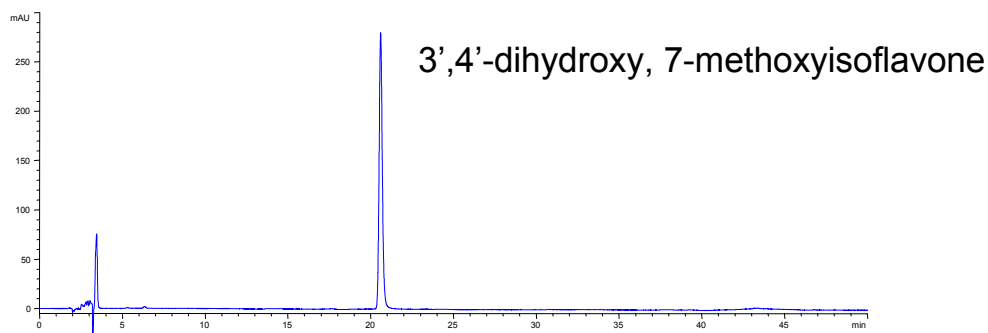
7,3',4'-trihydroxyisoflavone



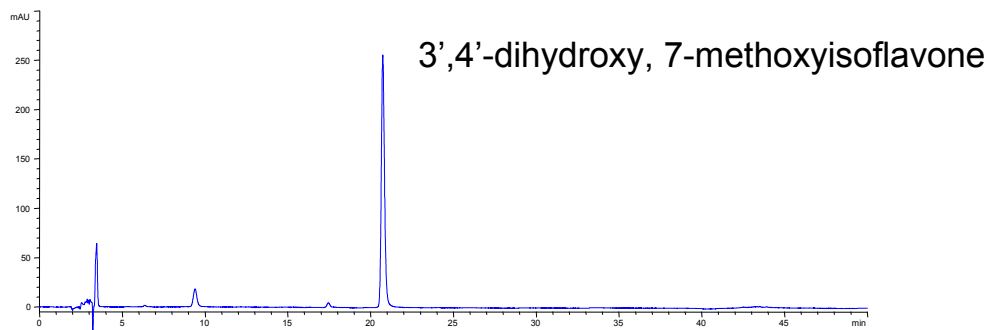
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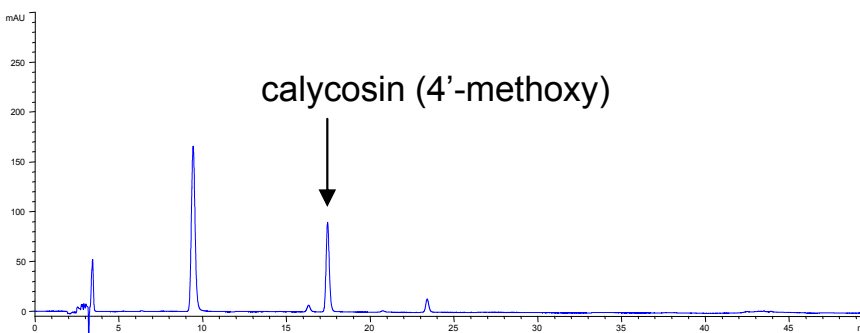
MtIOMT1



MtIOMT2



MtIOMT3



MtIOMT4

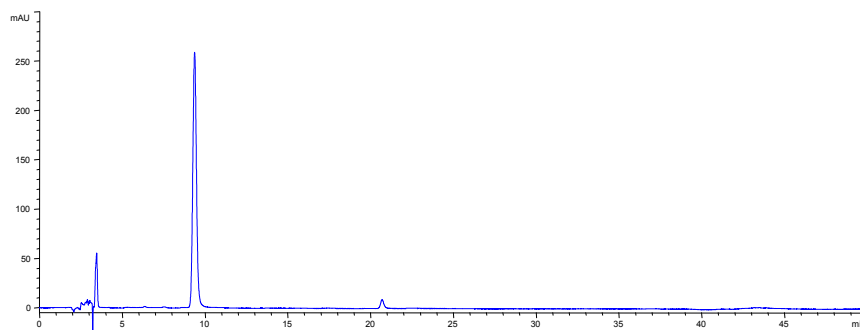
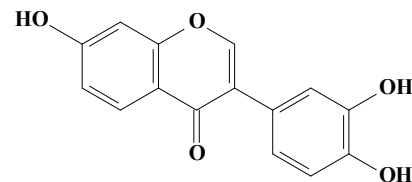
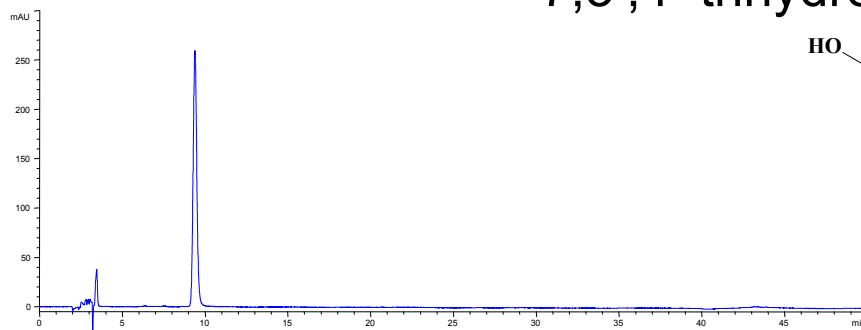


Figure S1(c). HPLC traces of reaction products with 7,3',4'-trihydroxyisoflavone

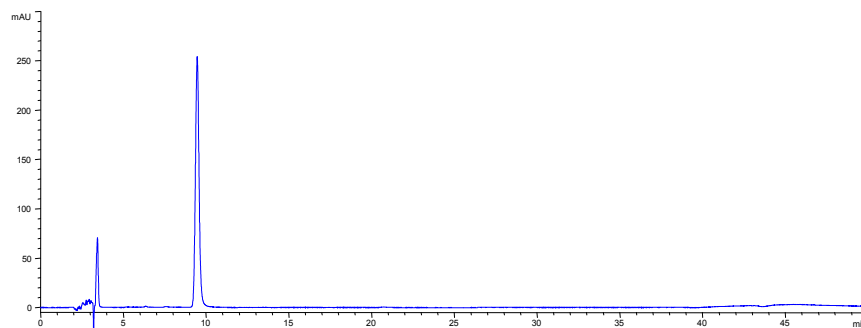
7,3',4'-trihydroxyisoflavone



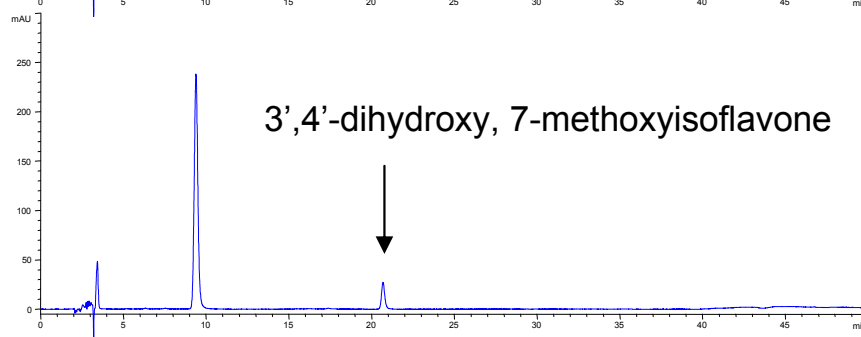
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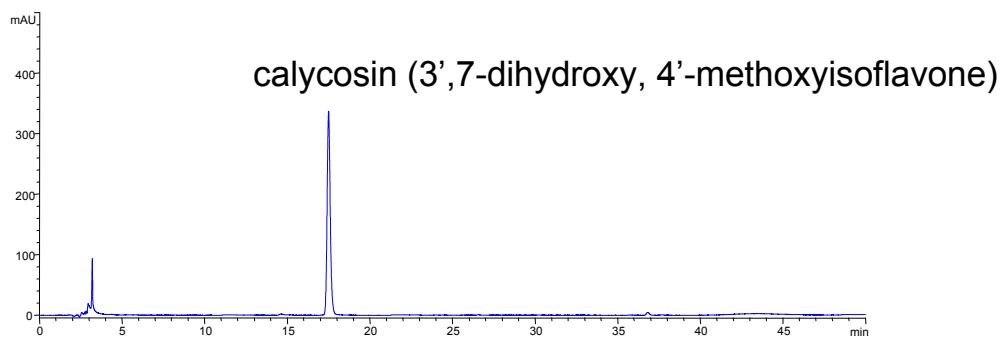
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MtIOMT7



Standard



Standard

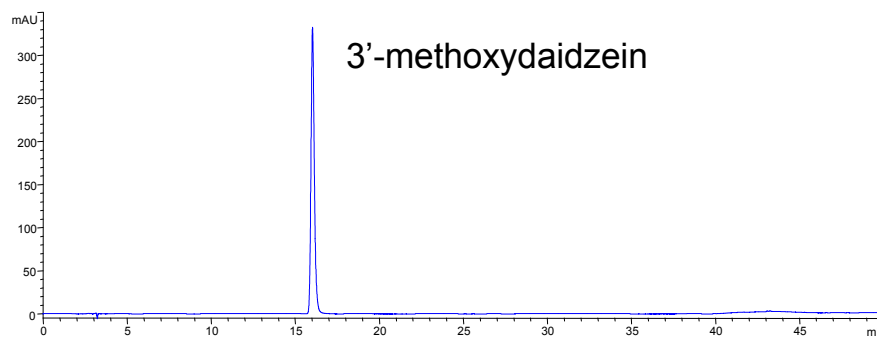
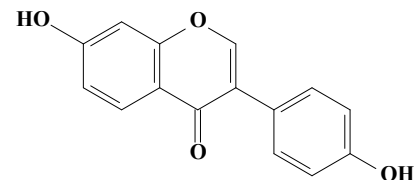
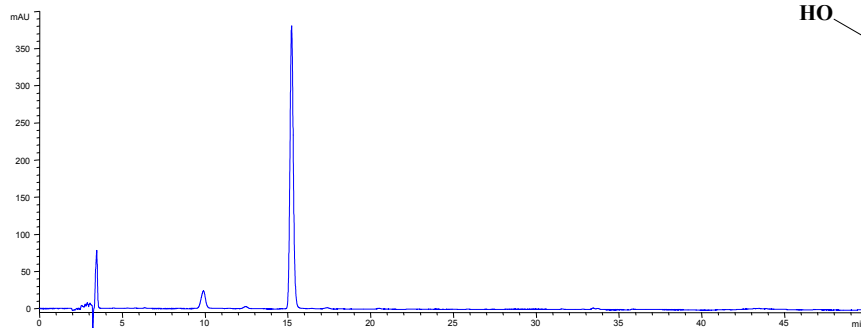


Figure S1(c), cont. HPLC traces of reaction products with 7,3',4'-trihydroxyisoflavone

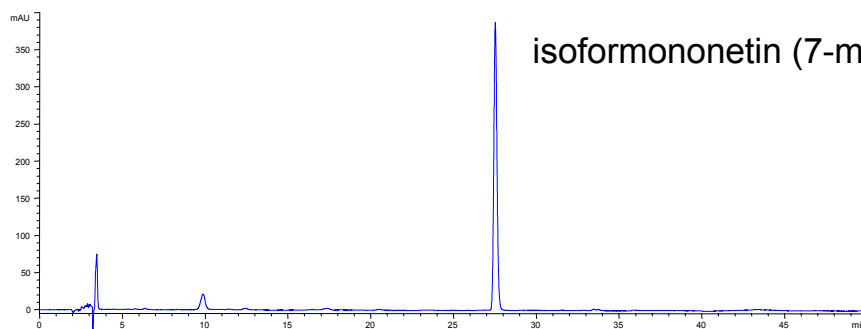
daidzein



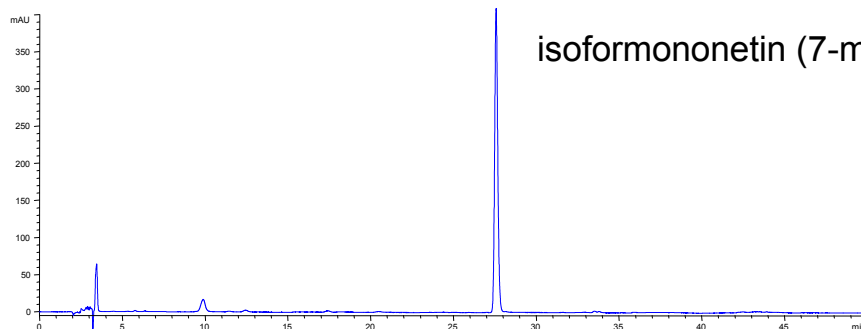
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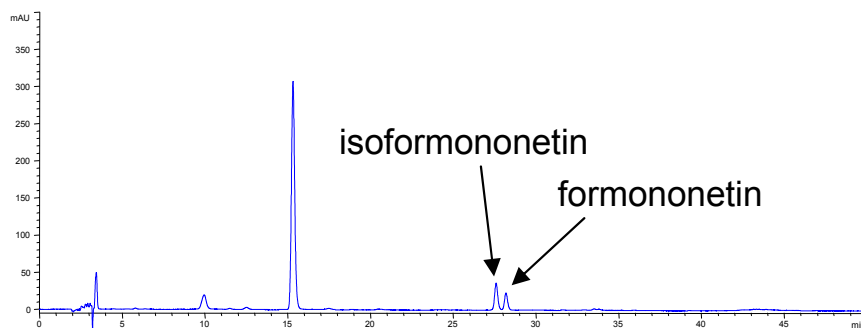
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MtIOMT2



MtIOMT3



MtIOMT4

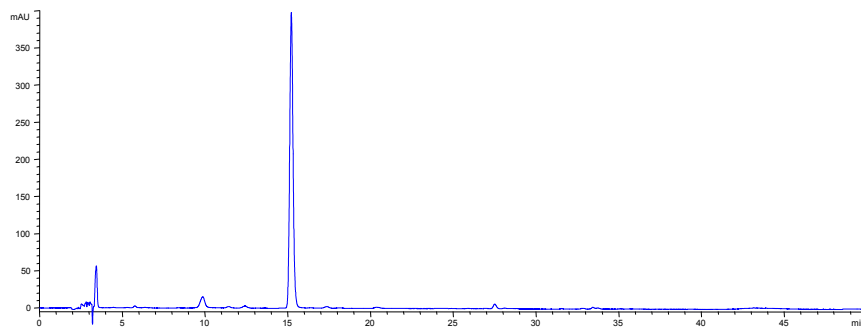
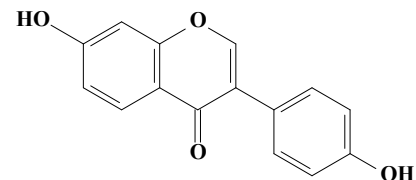
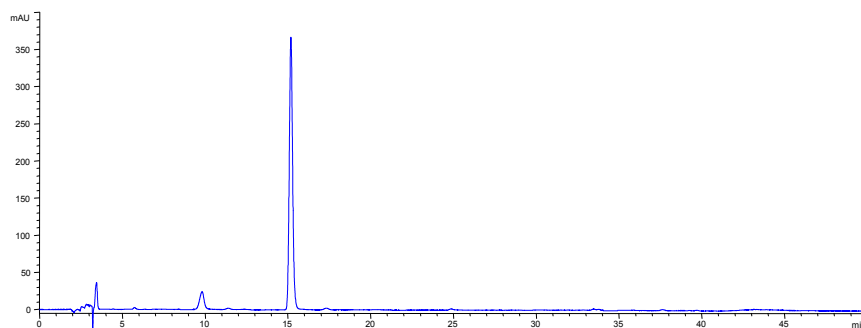


Figure S1(d). HPLC traces of reaction products with daidzein

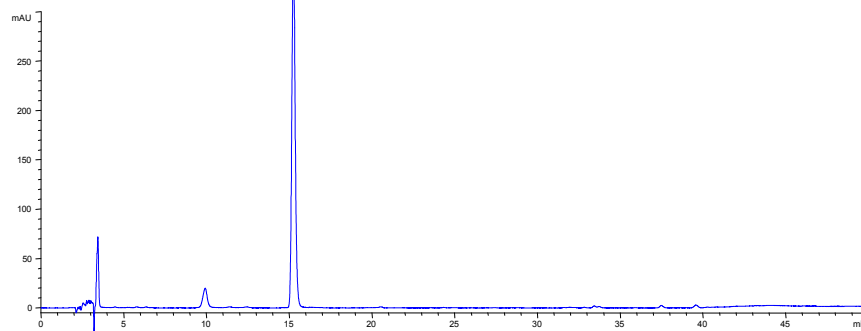
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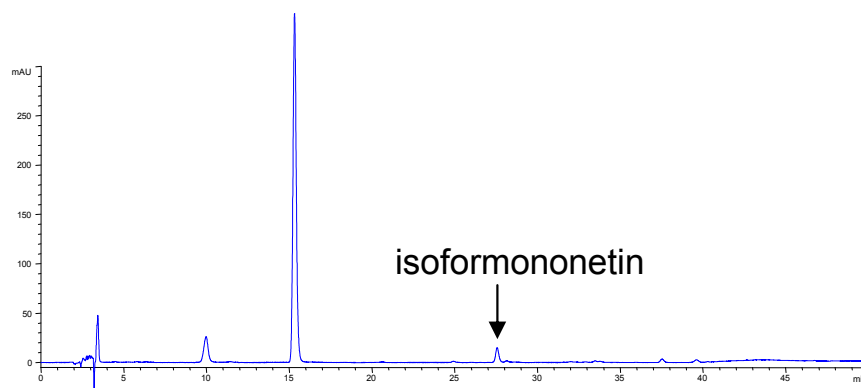
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MtIOMT6



MtIOMT7



Standards

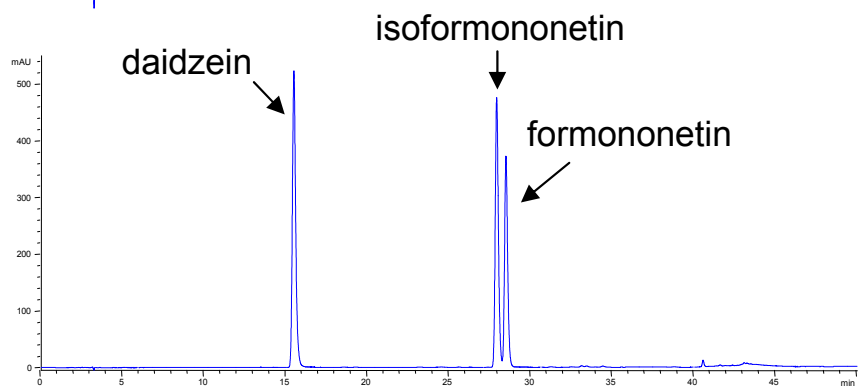


Figure S1(d), cont. HPLC traces of reaction products with daidzein

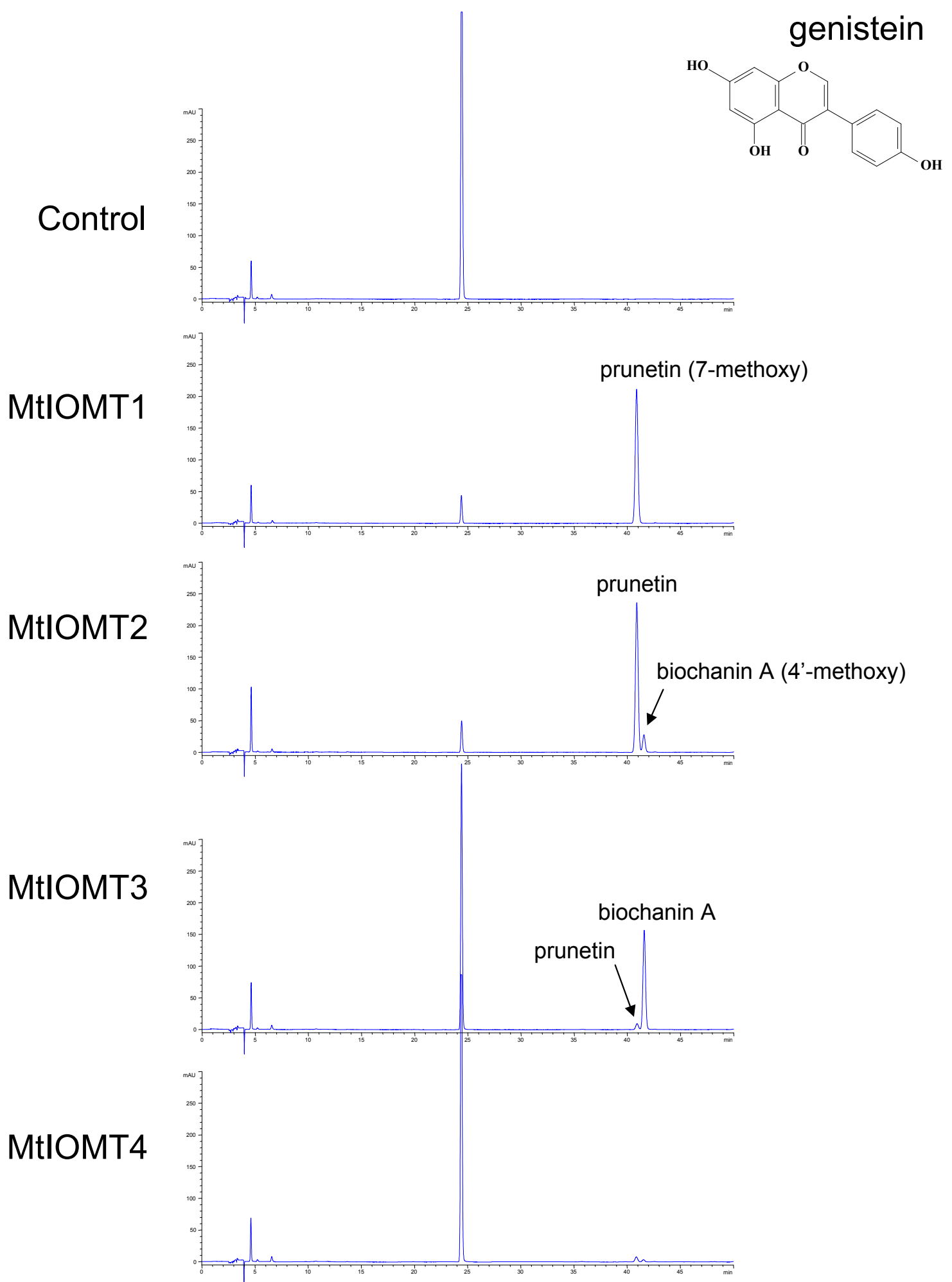
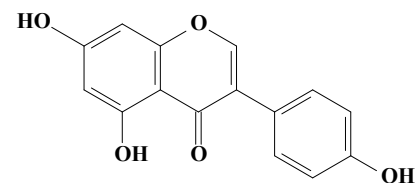
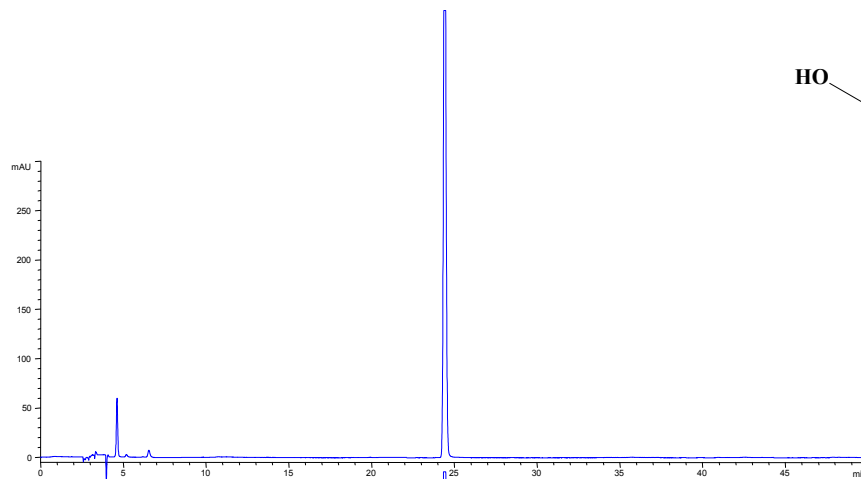


Figure S1(e). HPLC traces of reaction products with genistein

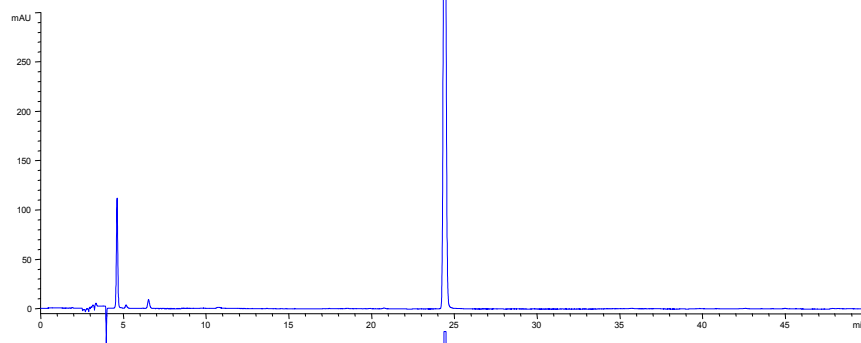
genistein



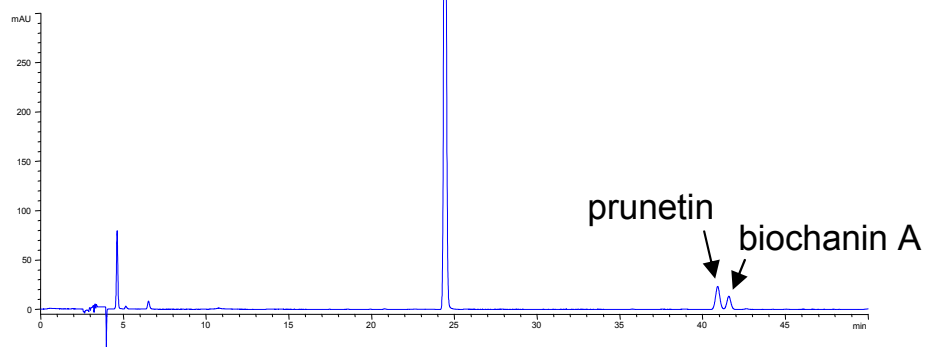
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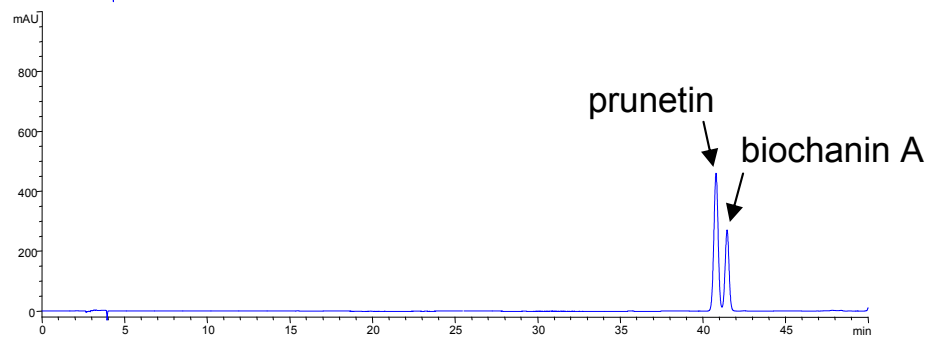
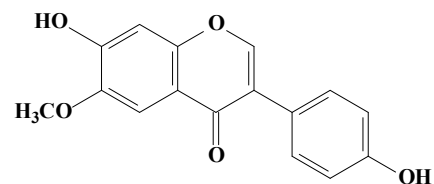
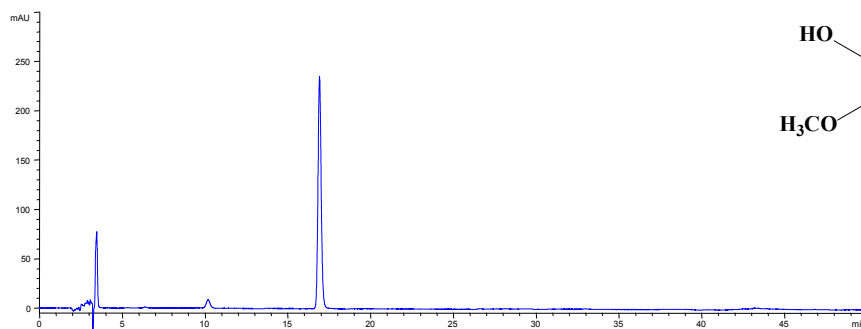


Figure S1(e), cont. HPLC traces of reaction products with genistein

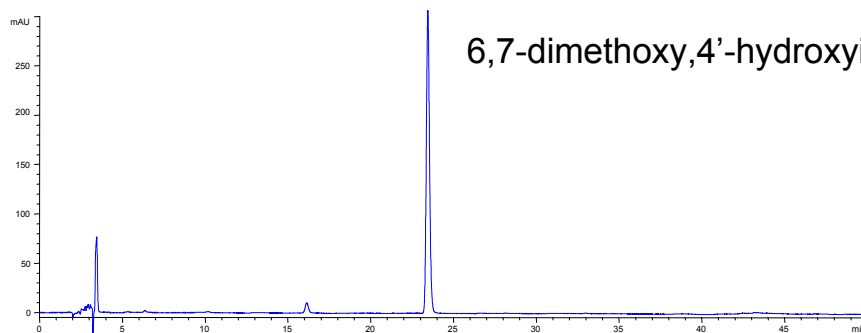
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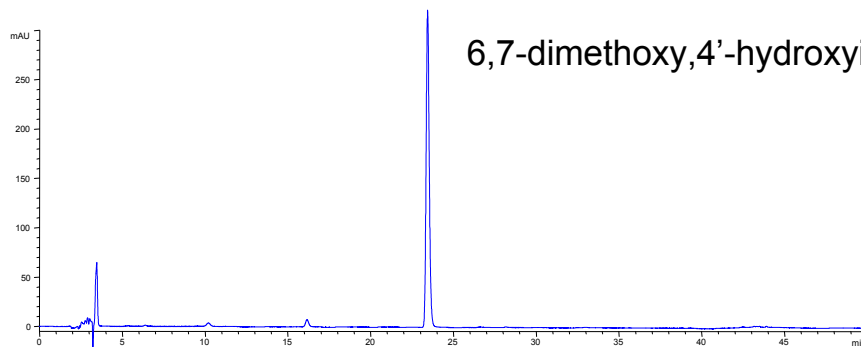
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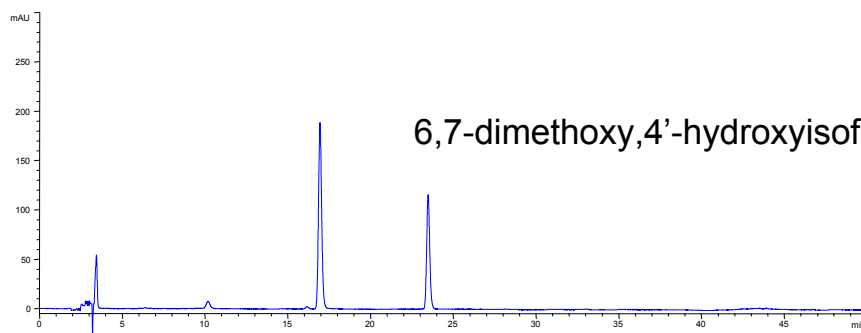
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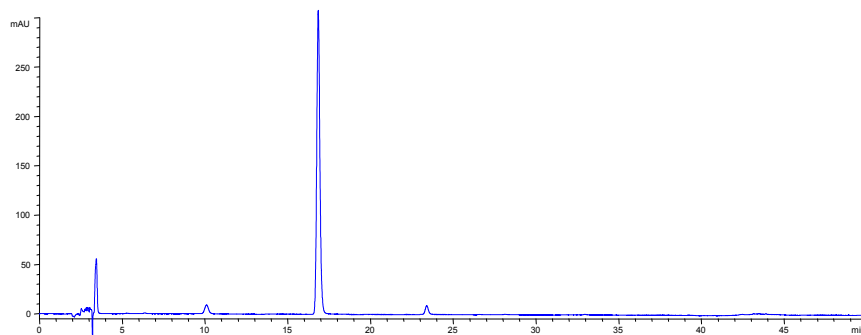
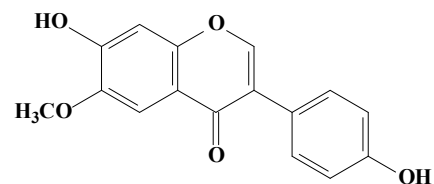
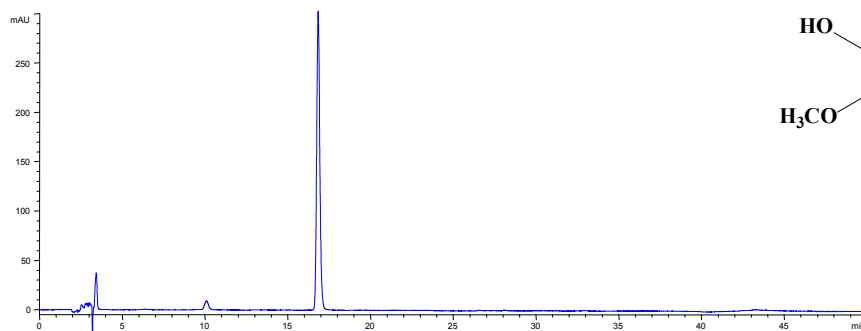


Figure S1(f). HPLC traces of reaction products with glycitein

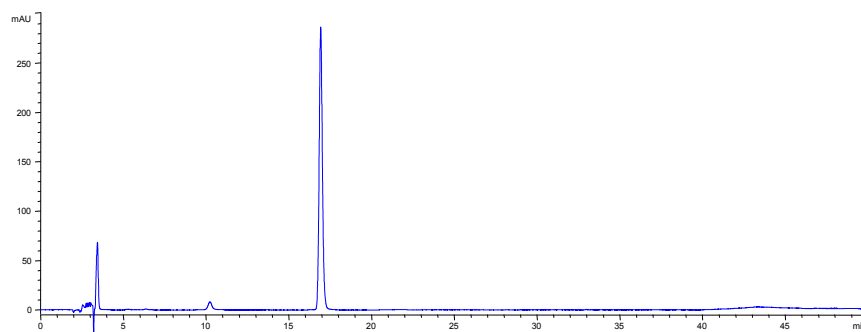
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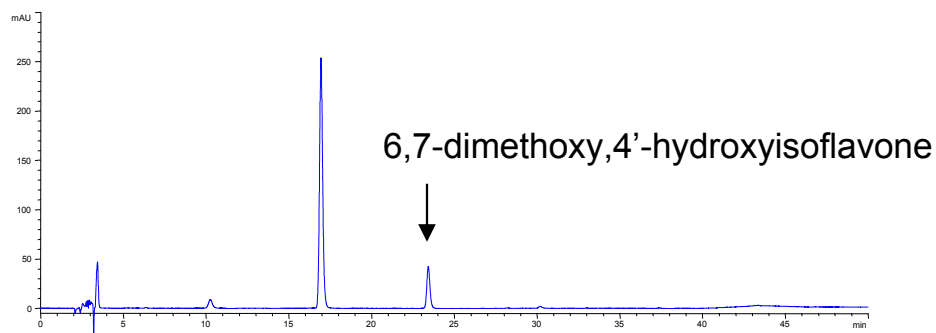
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MtIOMT7



Standard

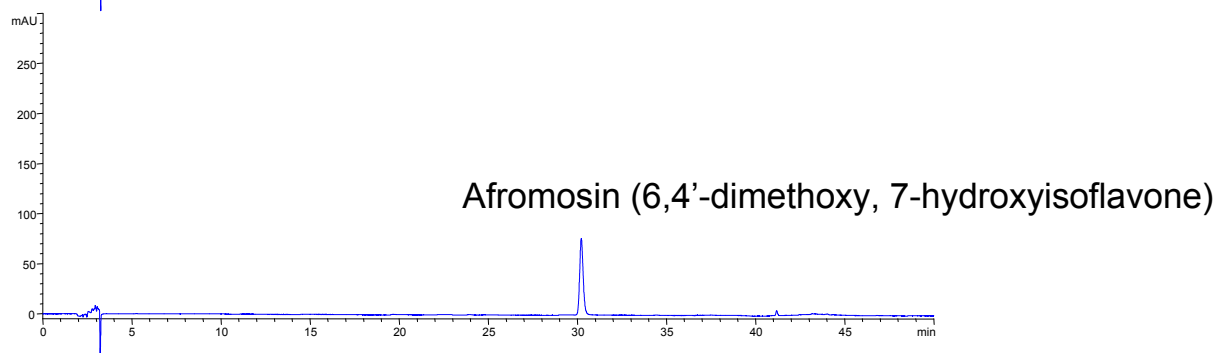


Figure S1(f), cont. HPLC traces of reaction products with glycitein

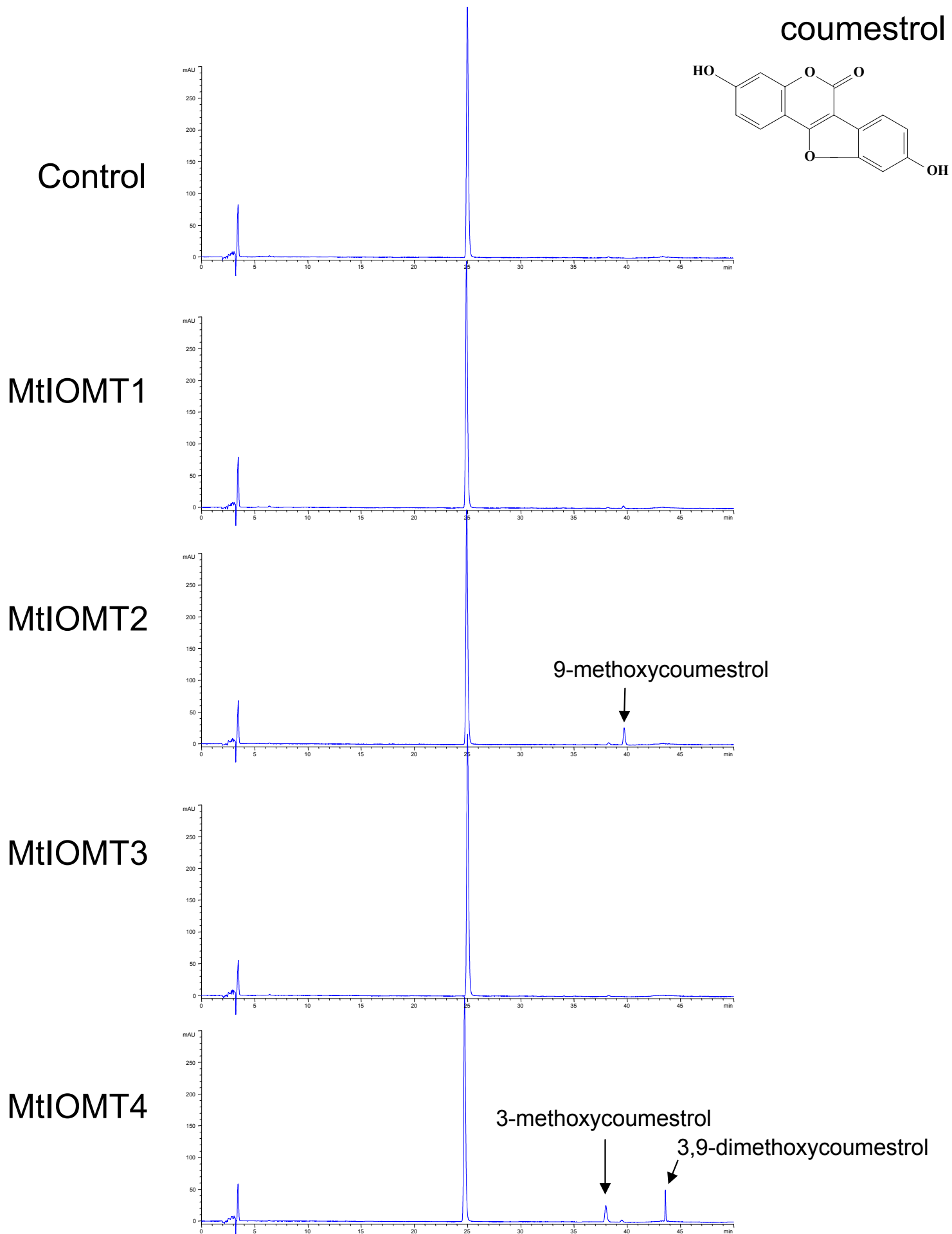
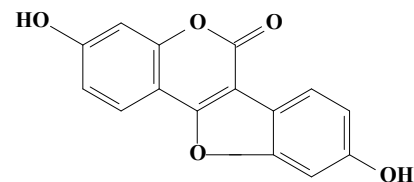
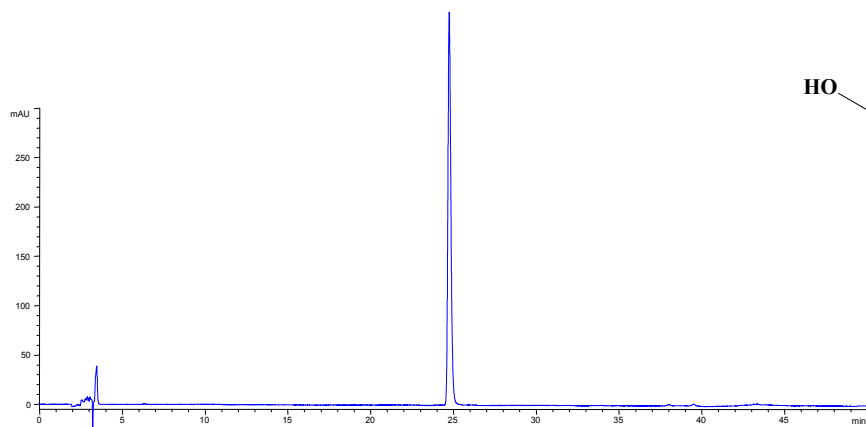


Figure S1(g). HPLC traces of reaction products with coumestrol

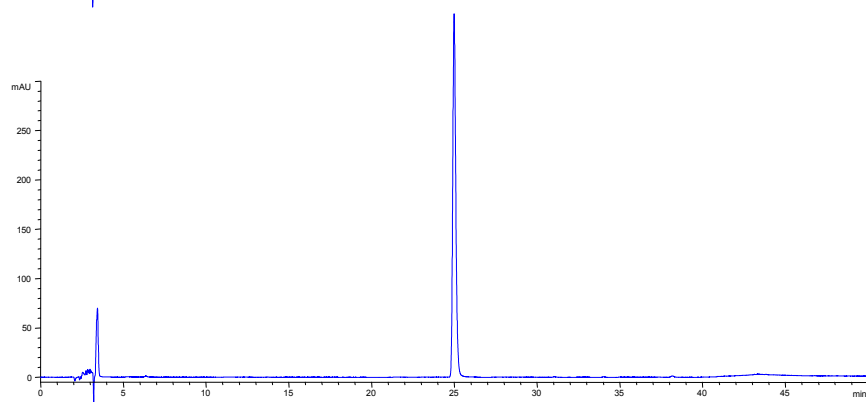
coumestrol



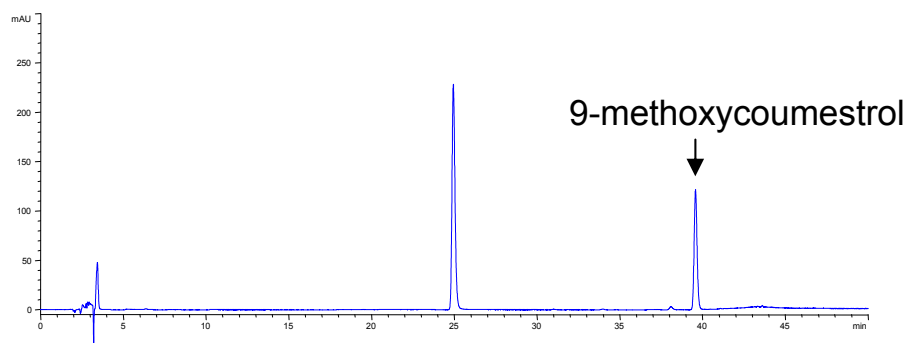
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MtIOMT7



Standard

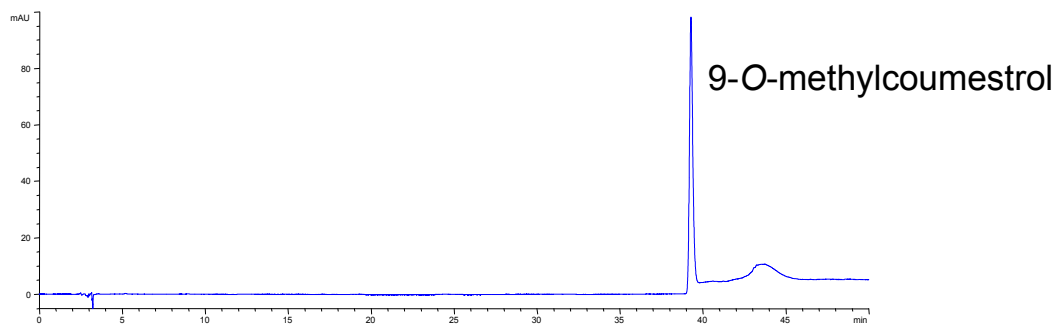
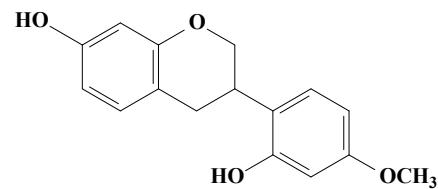
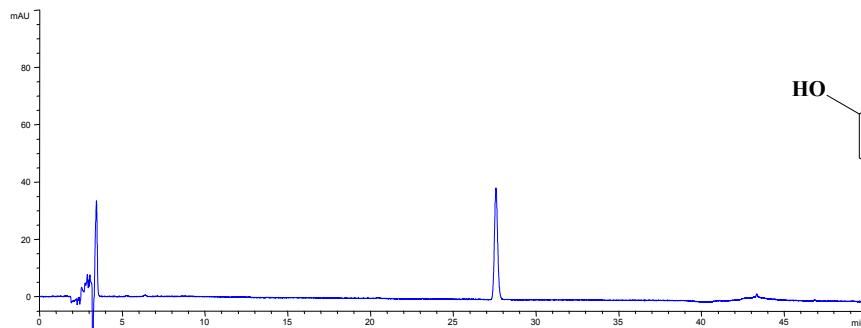


Figure S1(g), cont. HPLC traces of reaction products with coumestrol

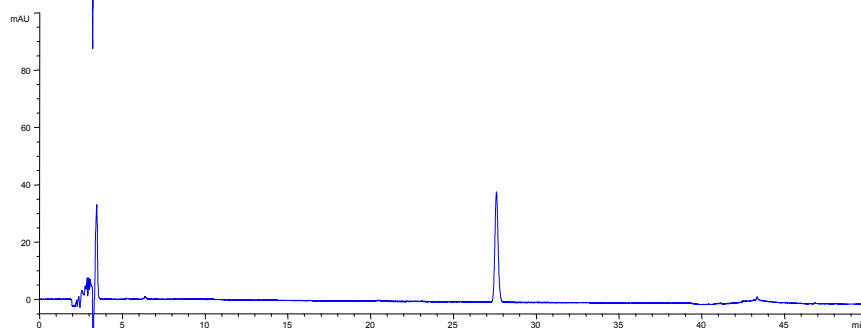
vestitol



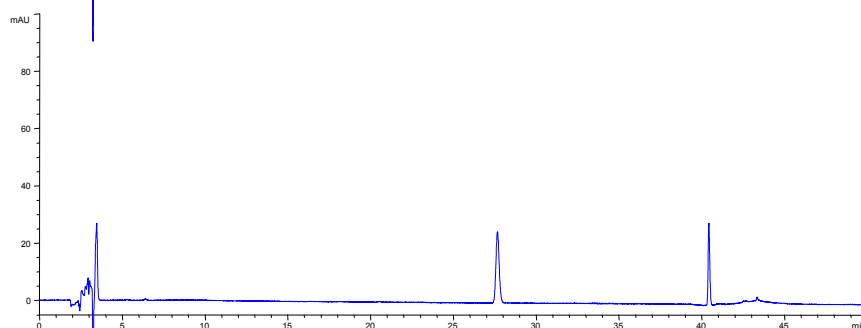
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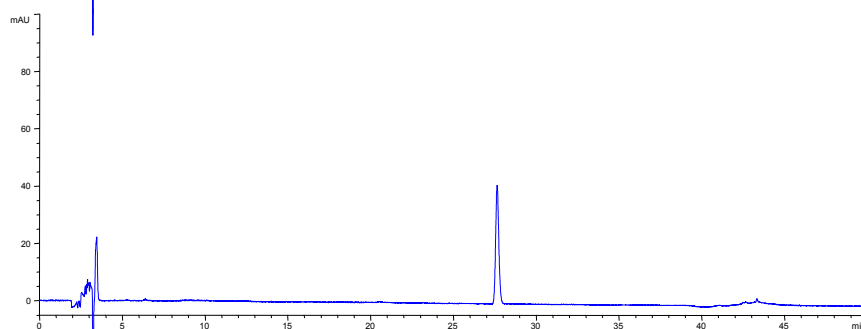
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MtIOMT2



MtIOMT3



MtIOMT4

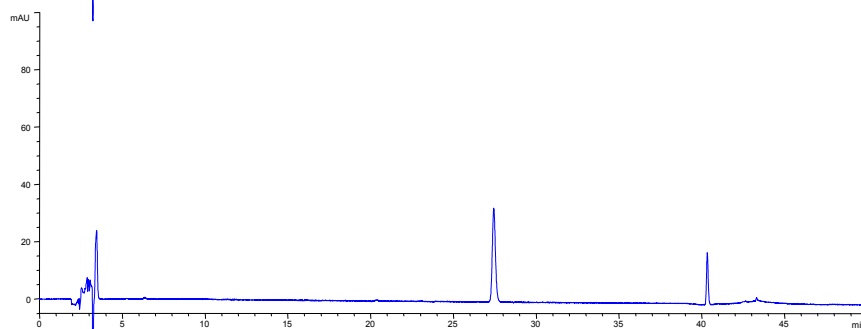
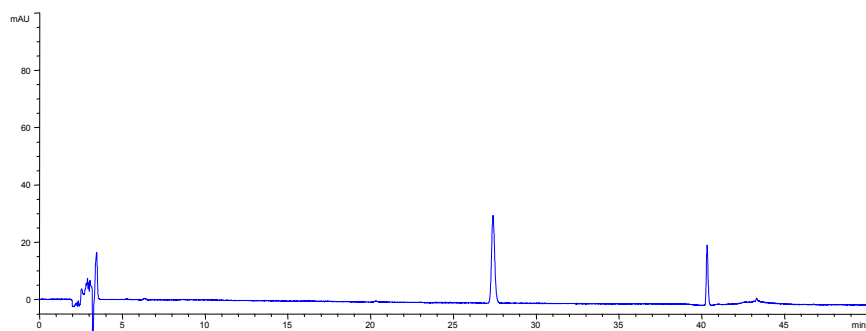
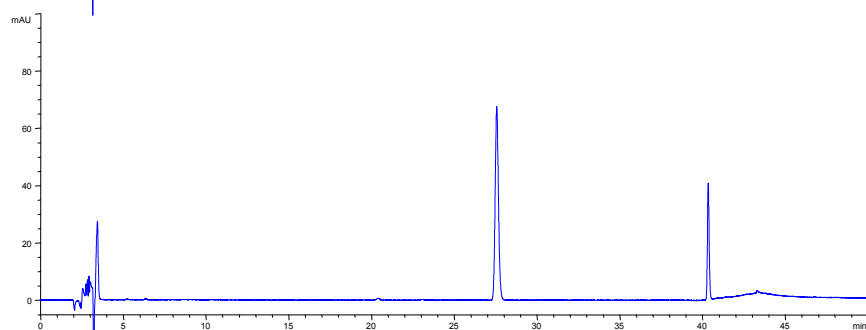


Figure S1(h). HPLC traces of reaction products with vestitol

MtlIOMT5



MtlIOMT6



MtlIOMT7

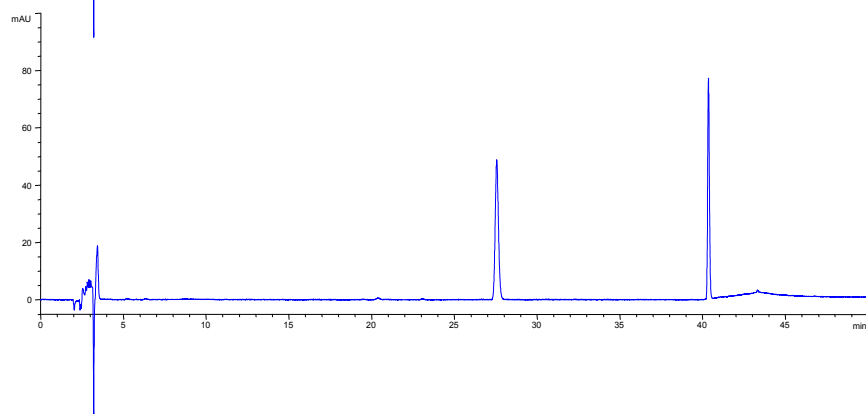
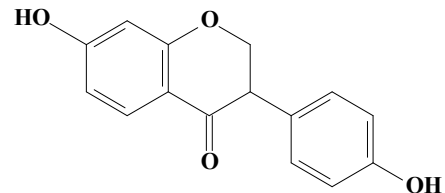
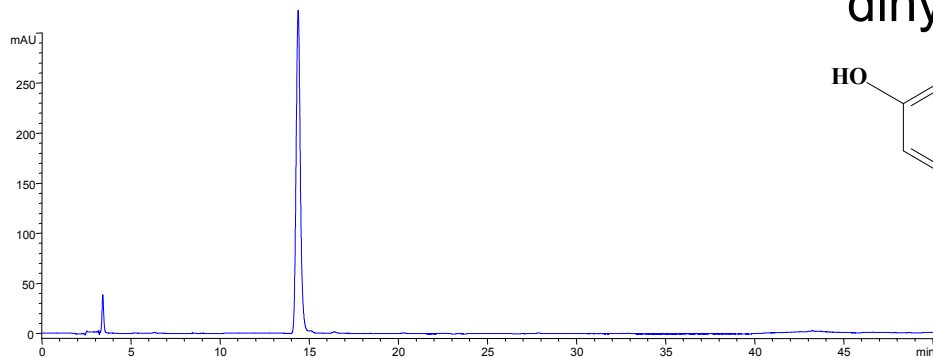


Figure S1(h), cont. HPLC traces of reaction products with vestitol

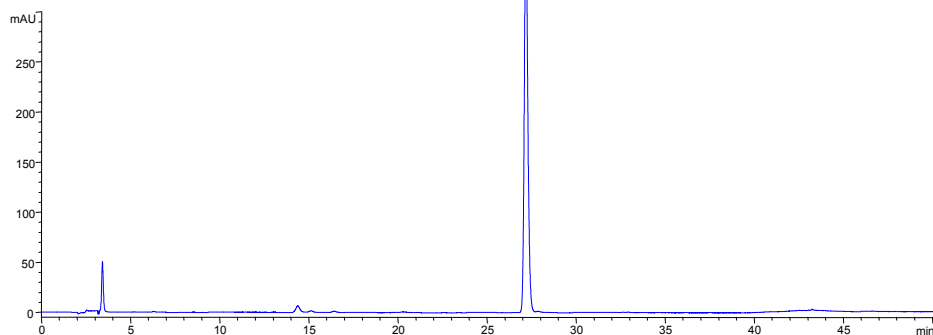
dihydrodaidzein



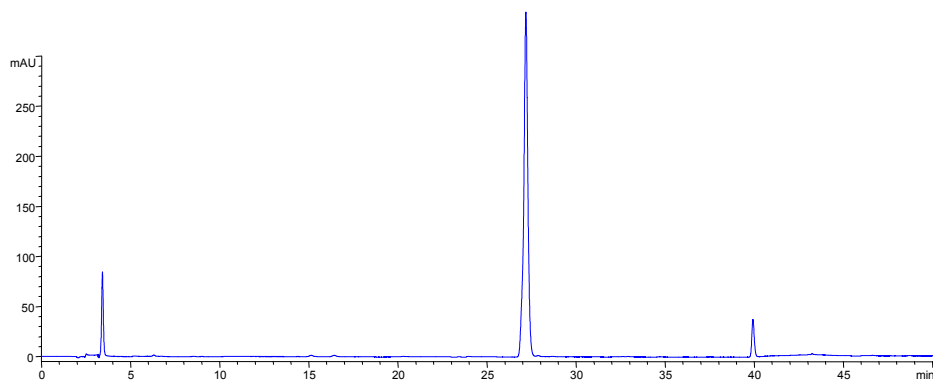
Control



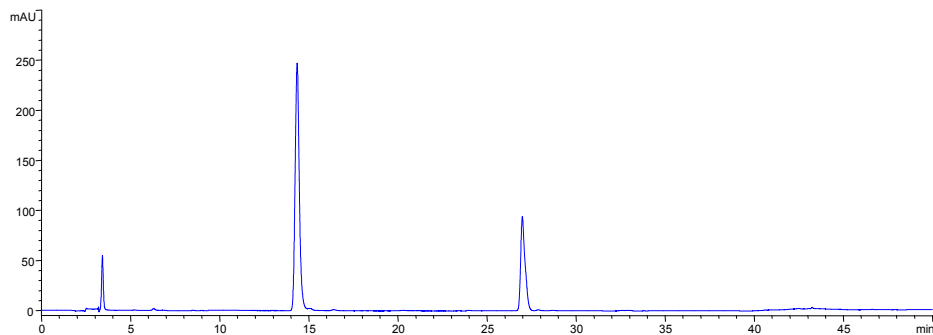
MtIOMT1



MtIOMT2



MtIOMT3



MtIOMT4

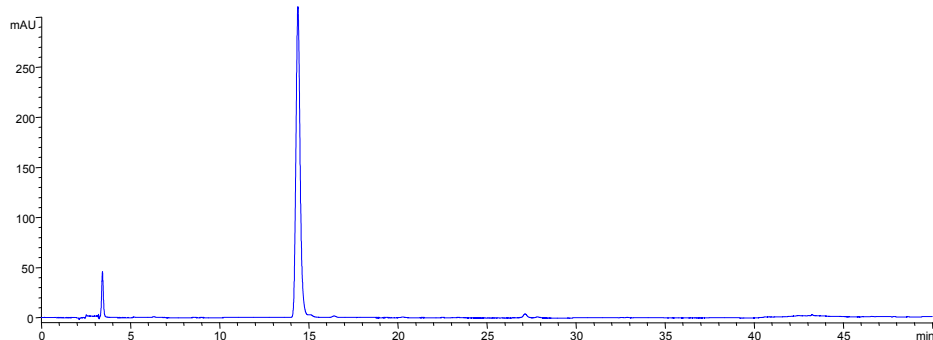
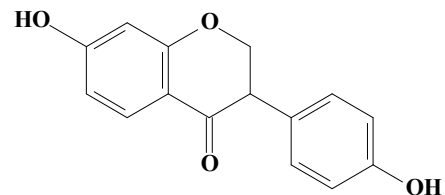
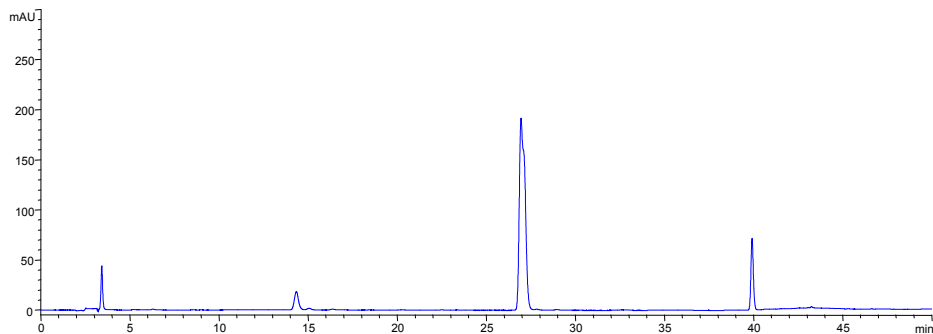


Figure S1(i). HPLC traces of reaction products with dihydrodaidzein

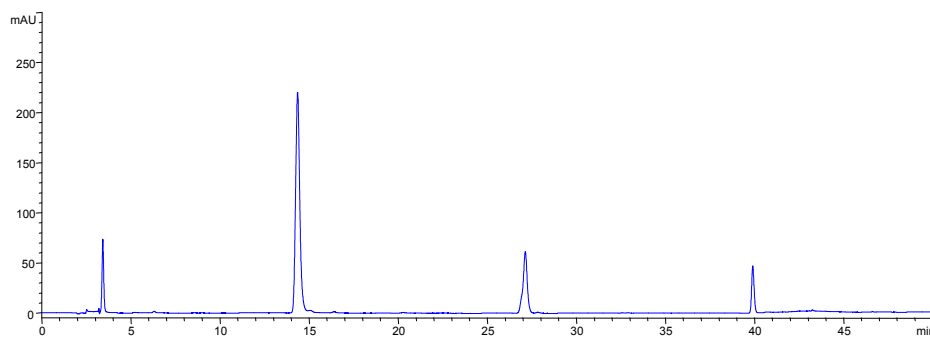
dihydrodaidzein



MtIOMT5



MtIOMT6



MtIOMT7

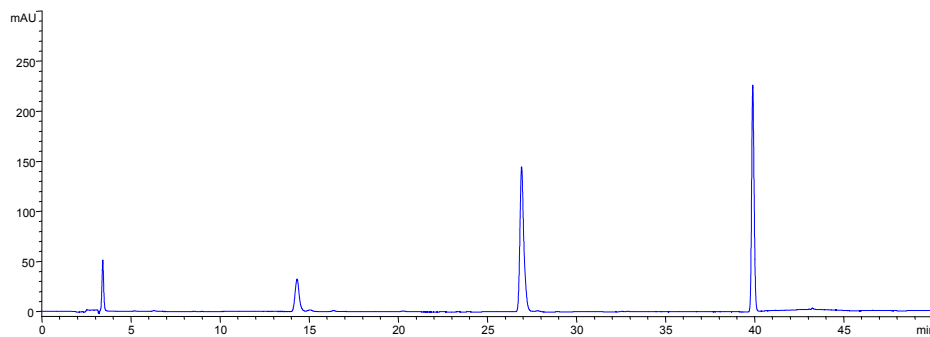
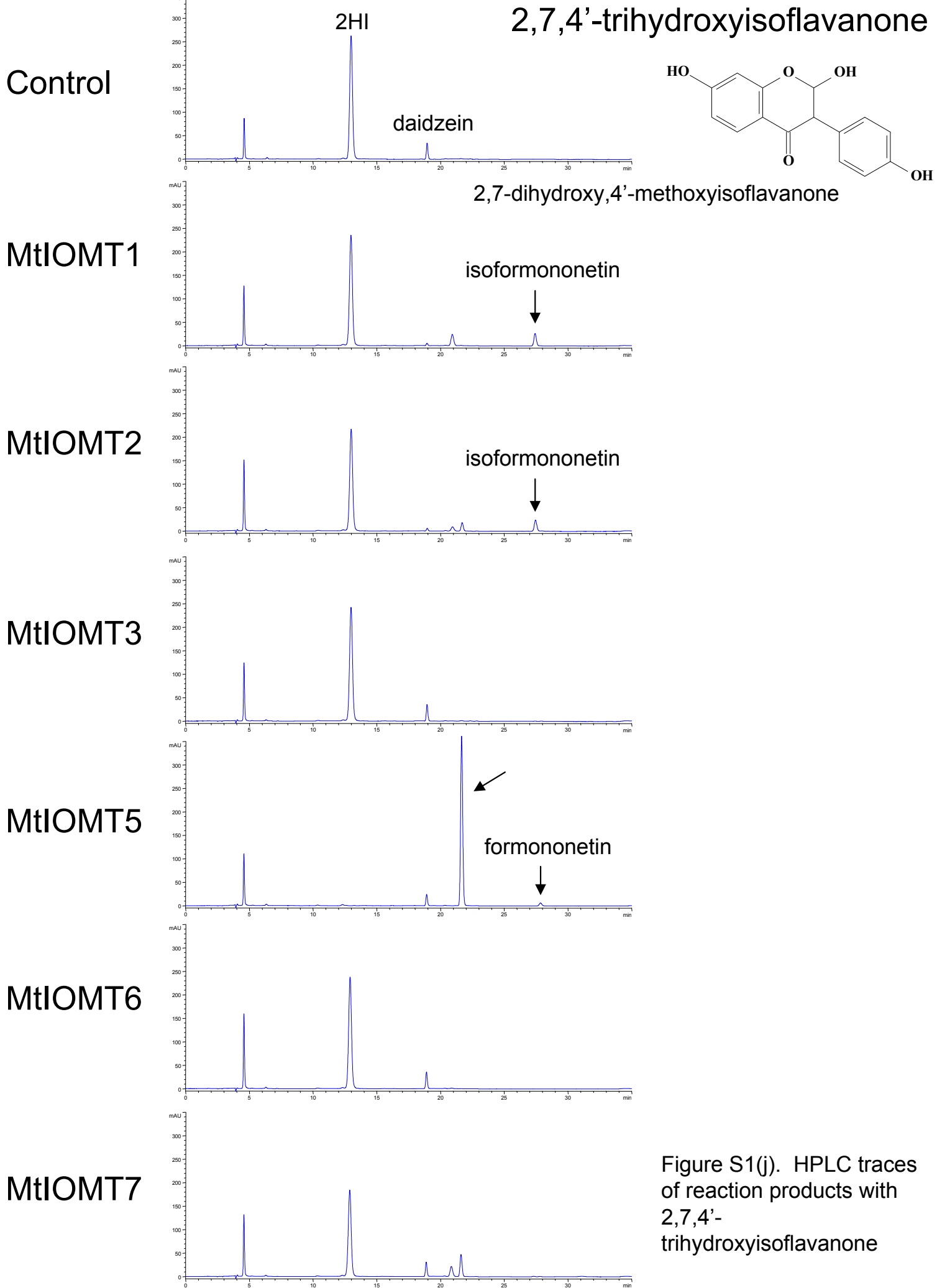


Figure S1(i), cont. HPLC traces of reaction products with dihydrodaidzein



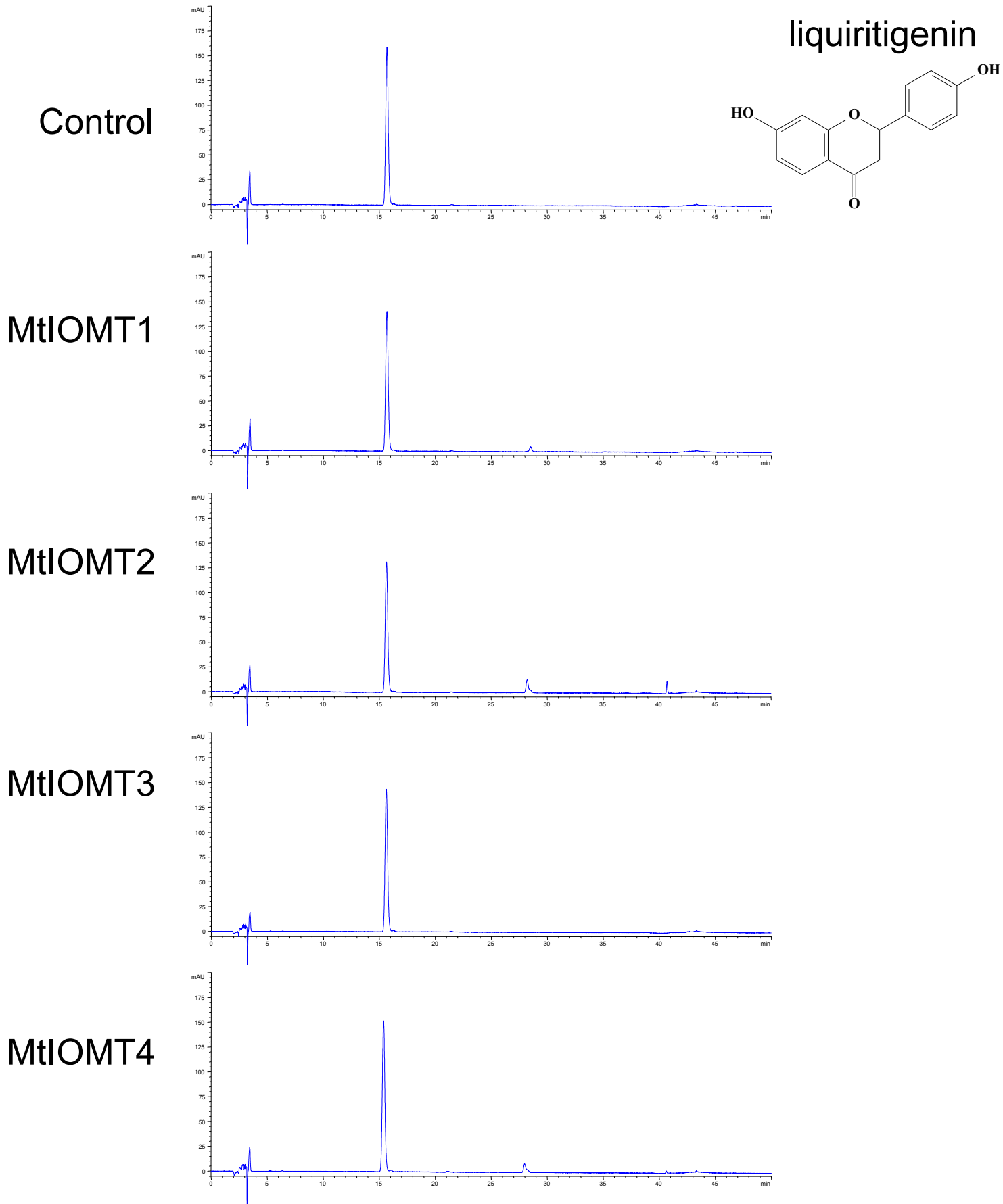
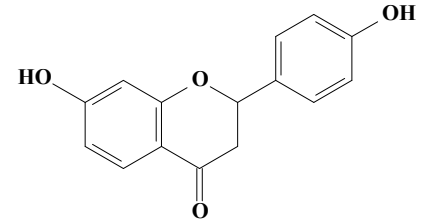
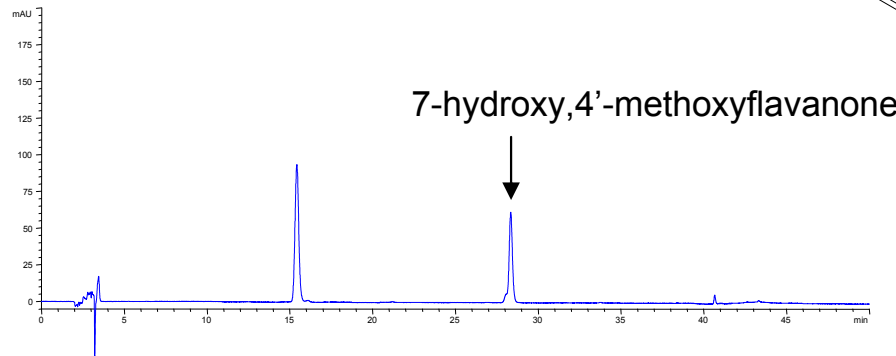


Figure S1(k). HPLC traces of reaction products with liquiritigenin

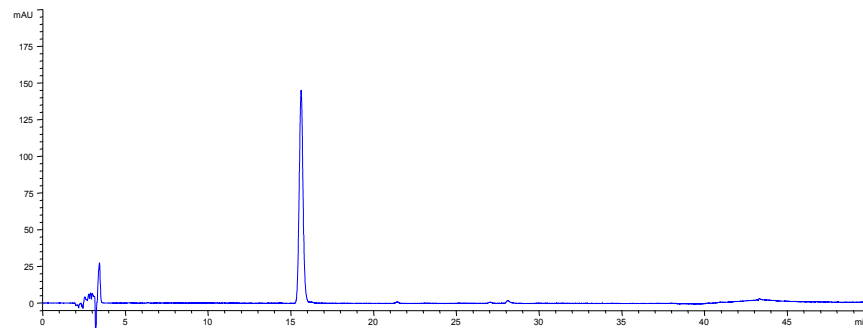
liquiritigenin



MtIOMT5



MtIOMT6



MtIOMT7

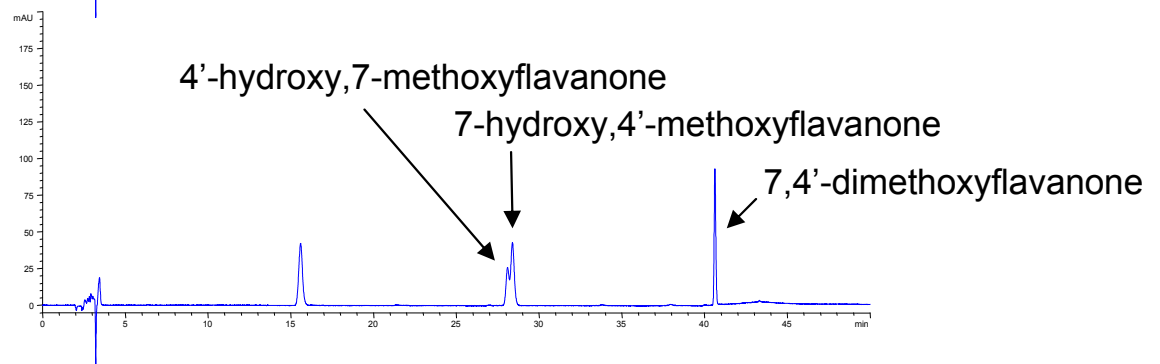
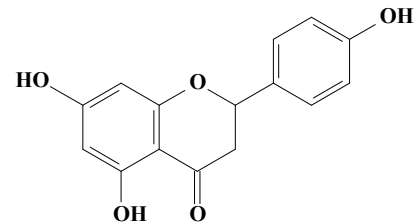
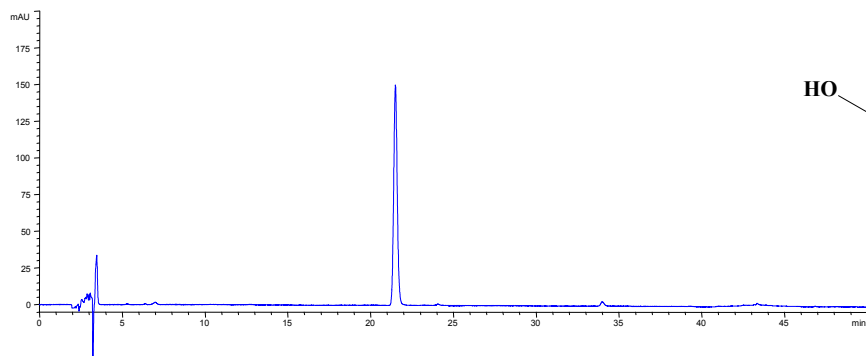


Figure S1(k), cont. HPLC traces of reaction products with liquiritigenin

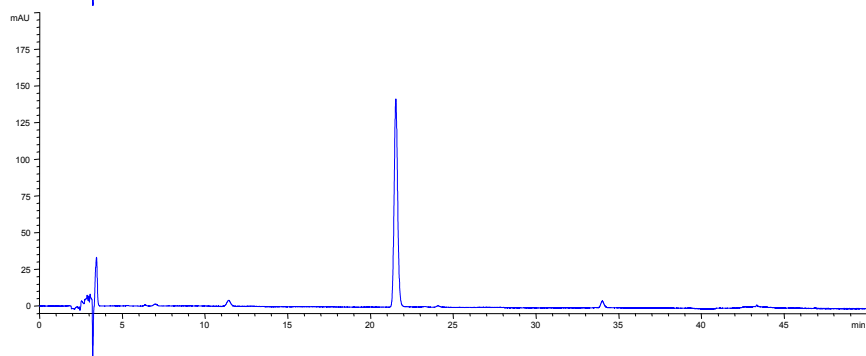
naringenin



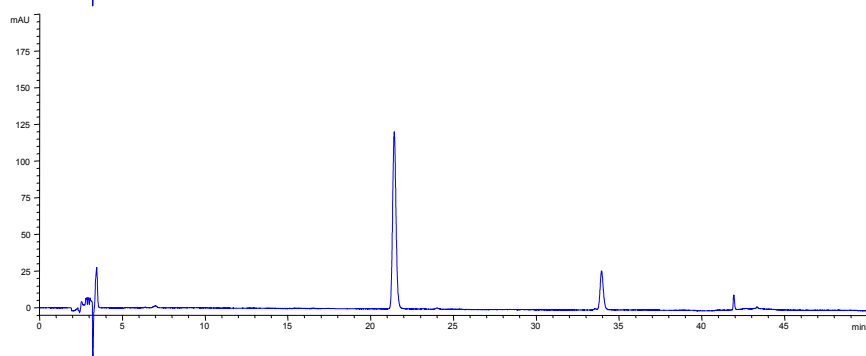
Control



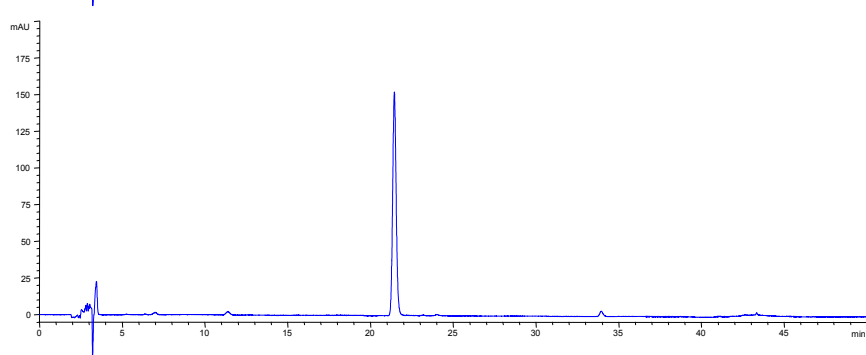
MtIOMT1



MtIOMT2



MtIOMT3



MtIOMT4

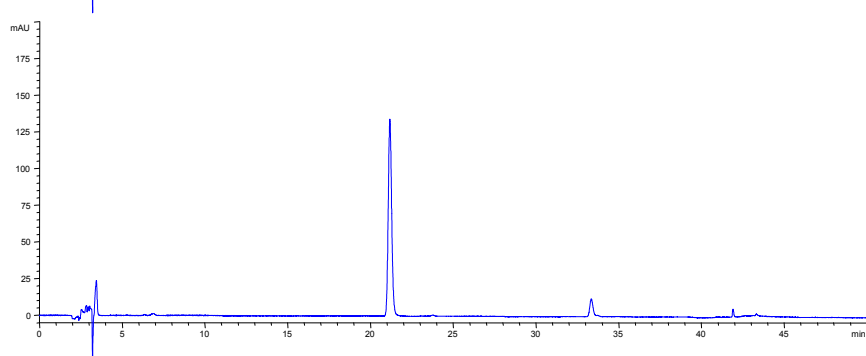
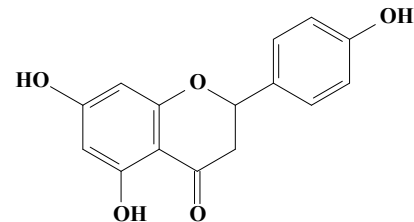
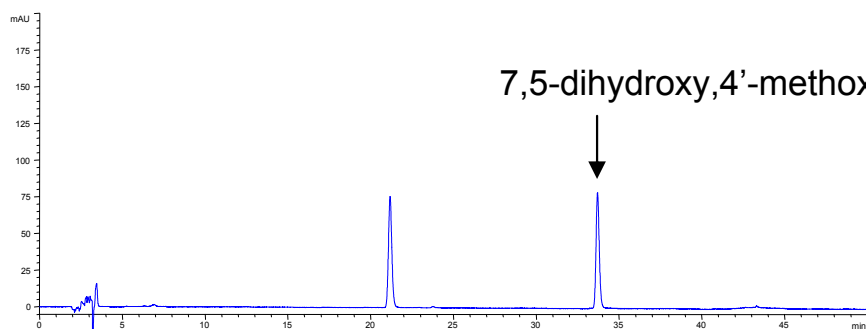


Figure S1(I). HPLC traces of reaction products with naringenin

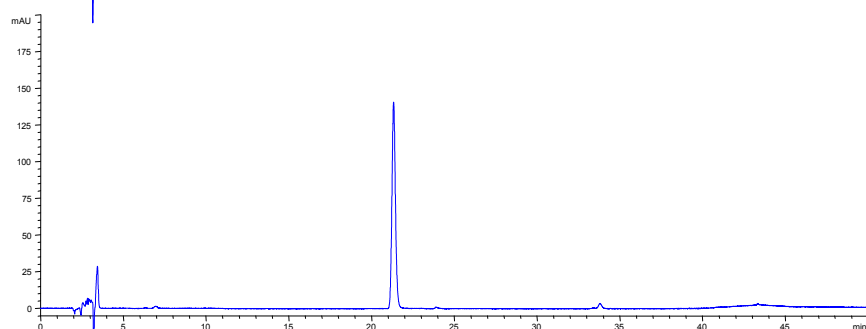
naringenin



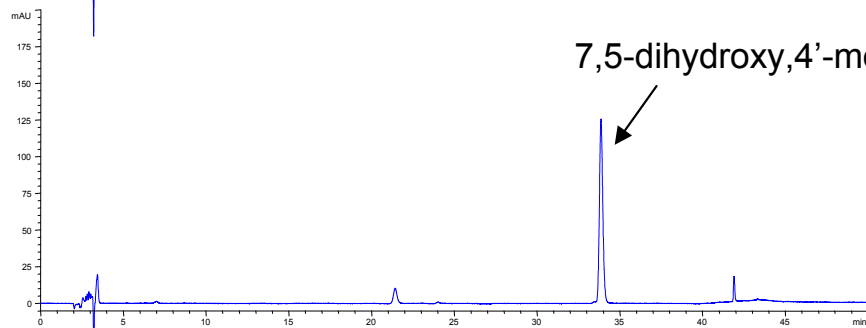
MtIOMT5



MtIOMT6



MtIOMT7



Standard

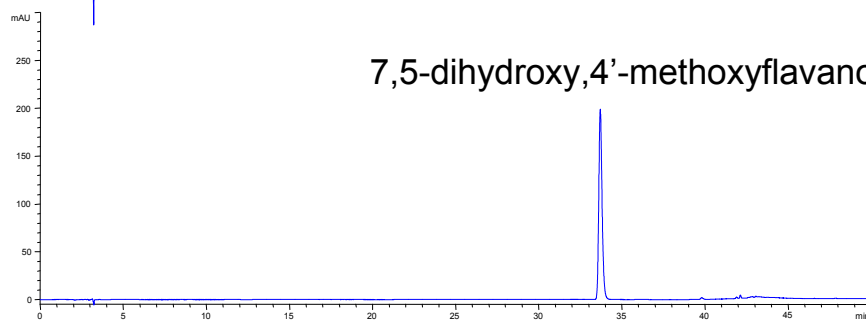
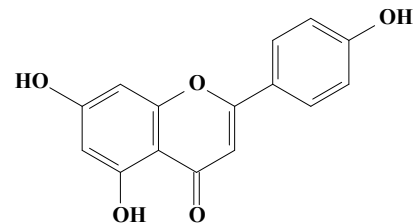
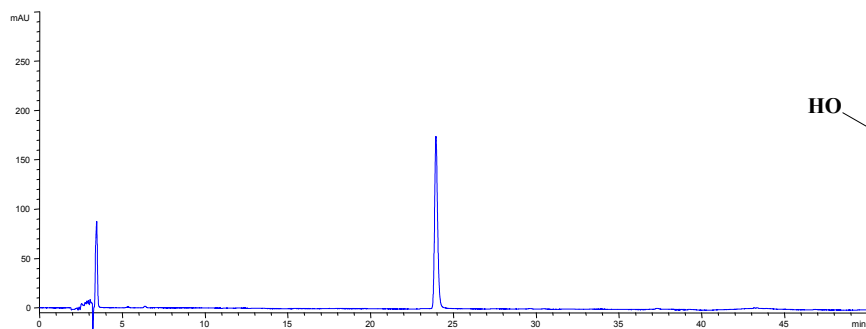


Figure S1(l), cont. HPLC traces of reaction products with naringenin

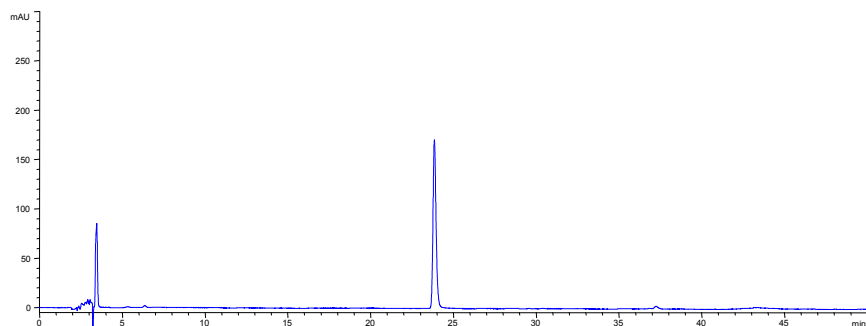
apigenin



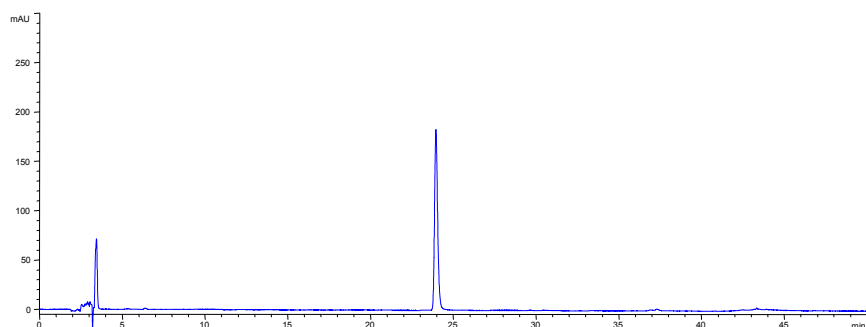
Control



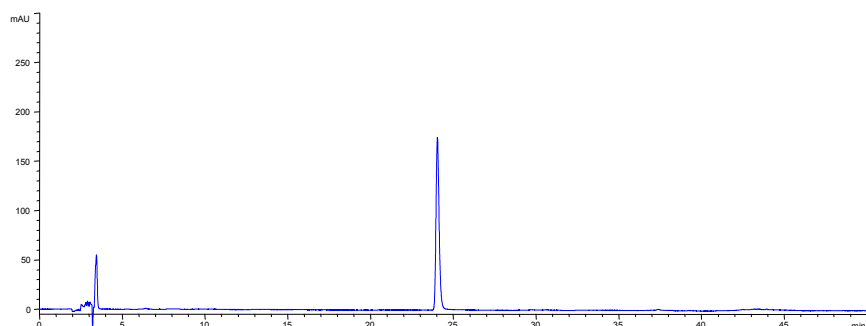
MtIOMT1



MtIOMT2



MtIOMT3



MtIOMT4

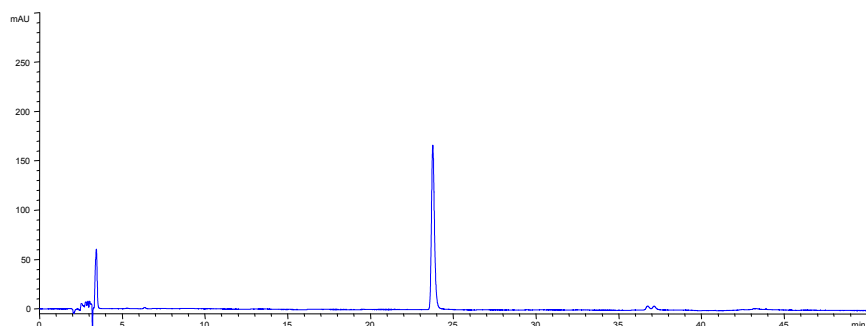
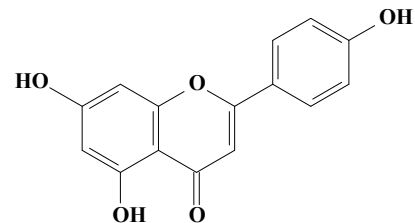
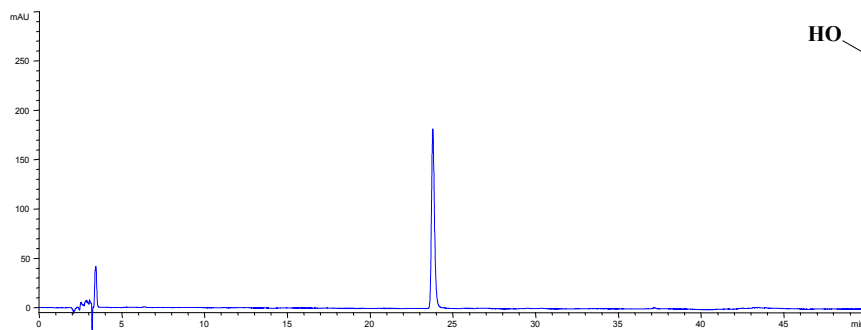


Figure S1(m). HPLC traces of reaction products with apigenin

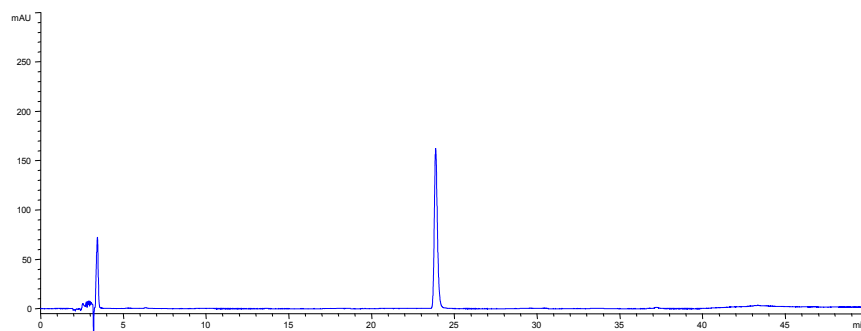
apigenin



MtIOMT5



MtIOMT6



MtIOMT7

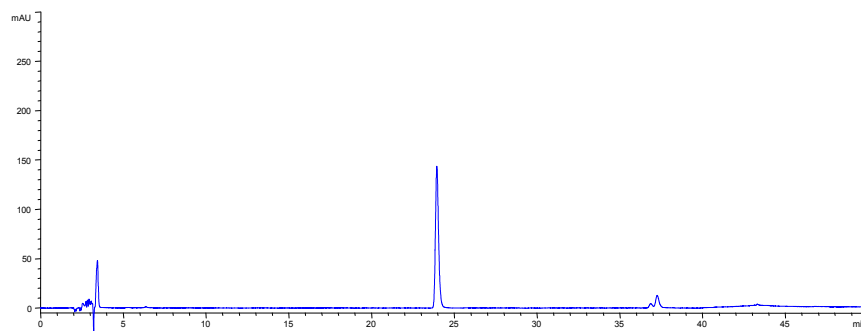
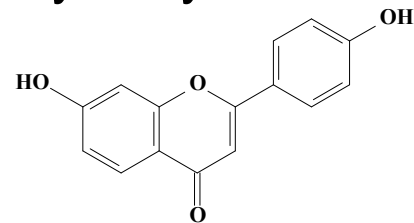
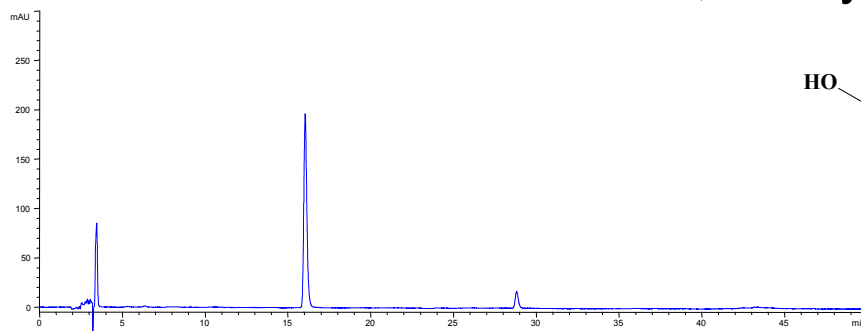


Figure S1(m), cont. HPLC traces of reaction products with apigenin

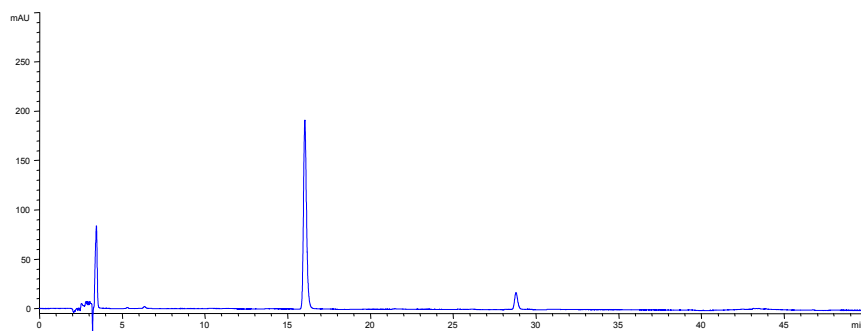
7,4'-dihydroxyflavone



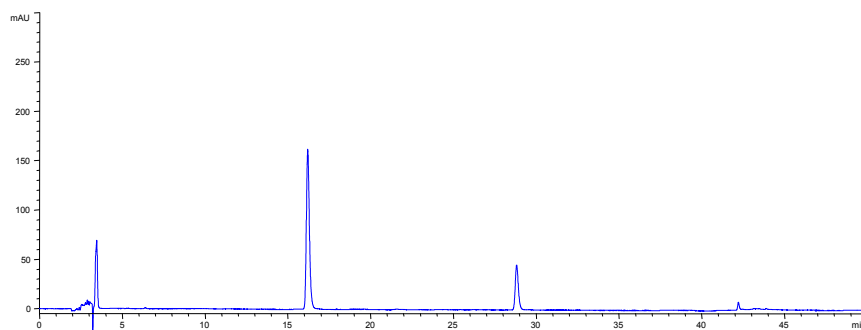
Control



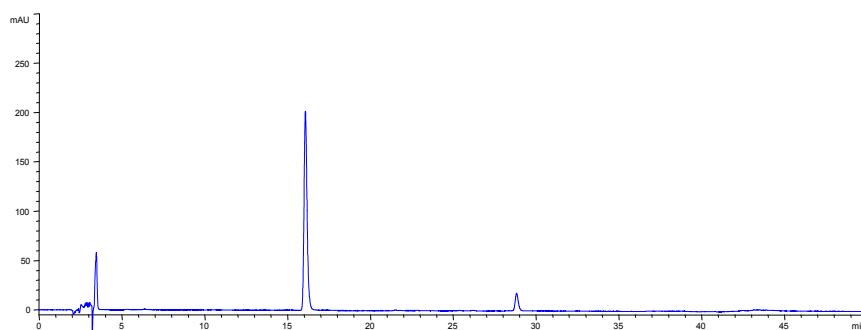
MtIOMT1



MtIOMT2



MtIOMT3



MtIOMT4

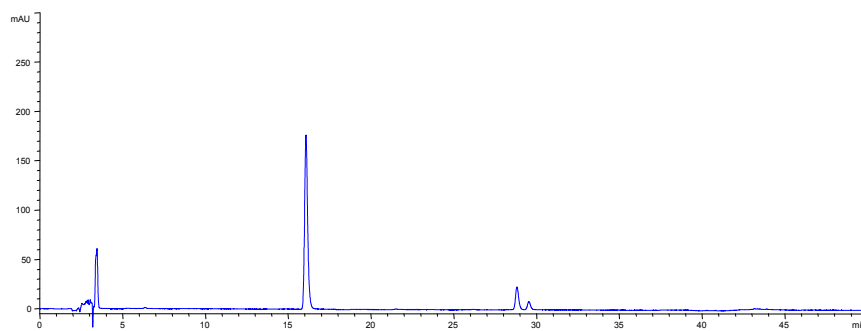
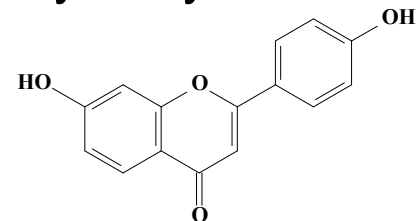
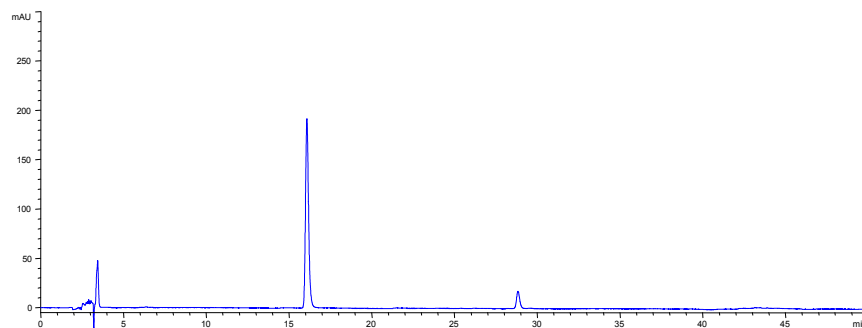


Figure S1(n). HPLC traces of reaction products with 7,4'-dihydroxyflavone

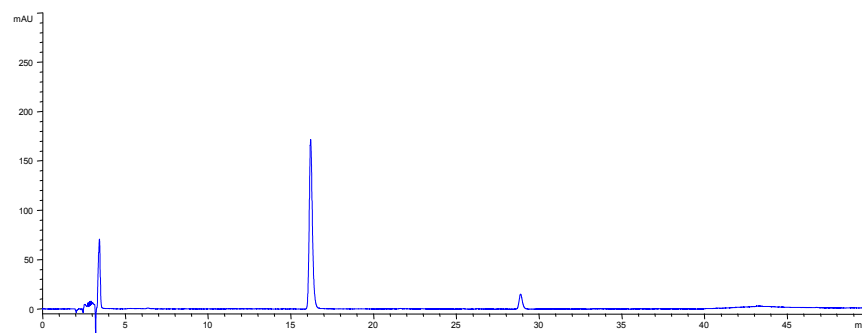
7,4'-dihydroxyflavone



MtIOMT5



MtIOMT6



MtIOMT7

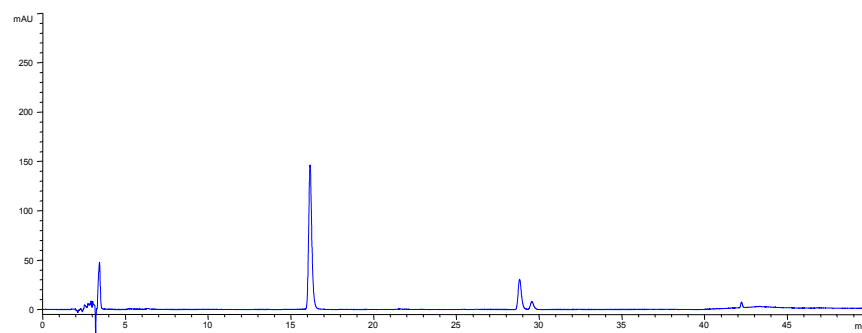


Figure S1(n), cont. HPLC traces of reaction products with 7,4'-dihydroxyflavone

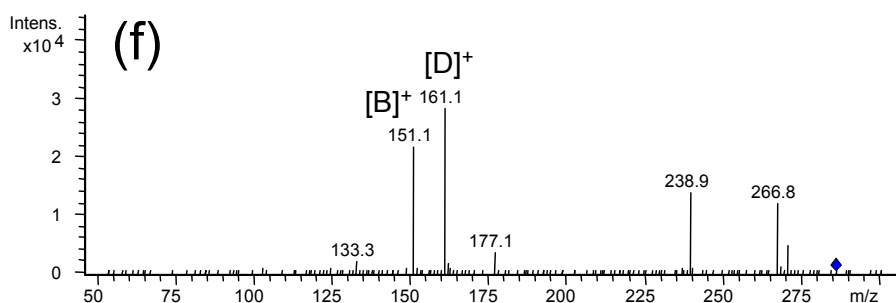
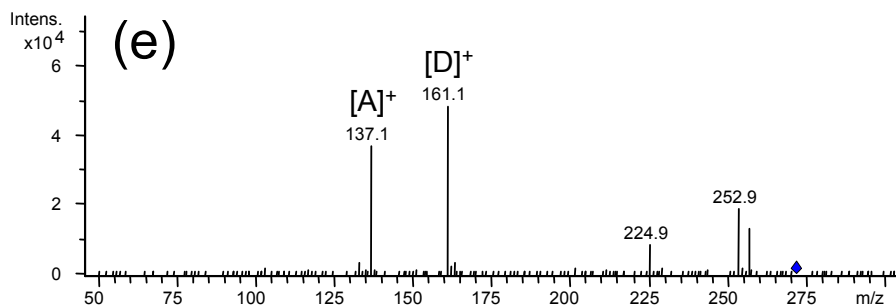
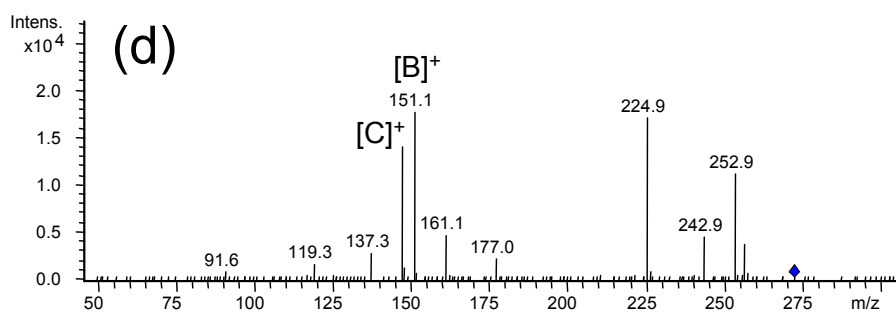
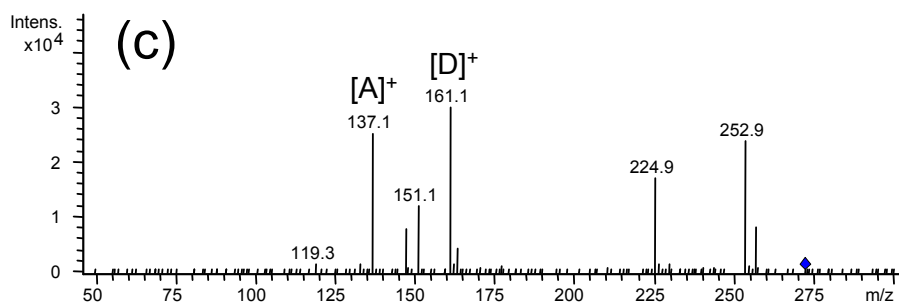
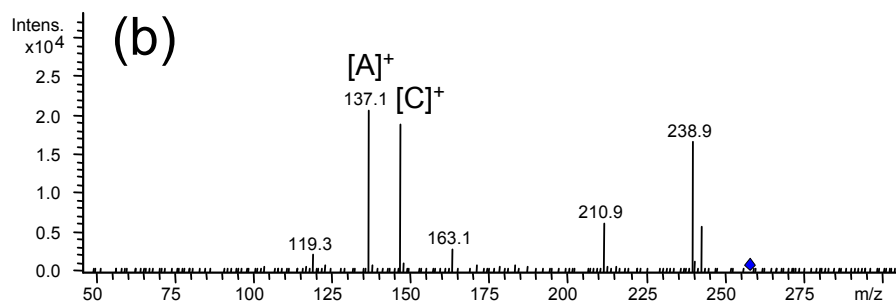
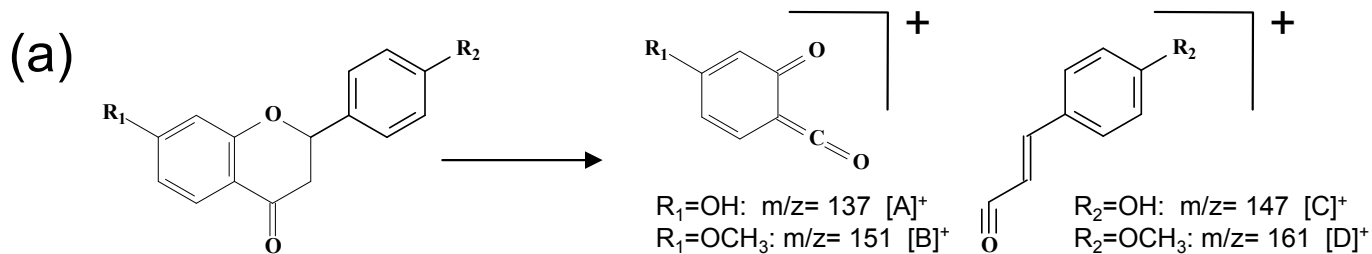


Figure S2. Positive ion LC/ESI/MSMS spectra of liquiritigenin reaction products. (a) Proposed fragmentation scheme. (b) Liquiritigenin, precursor ion is m/z 257. (c) MtlOMT5 methylated product, precursor ion is m/z 271. (d)-(f) MtlOMT7 methylated products, precursor ion is m/z 271 (d,e) or m/z 285 (f).

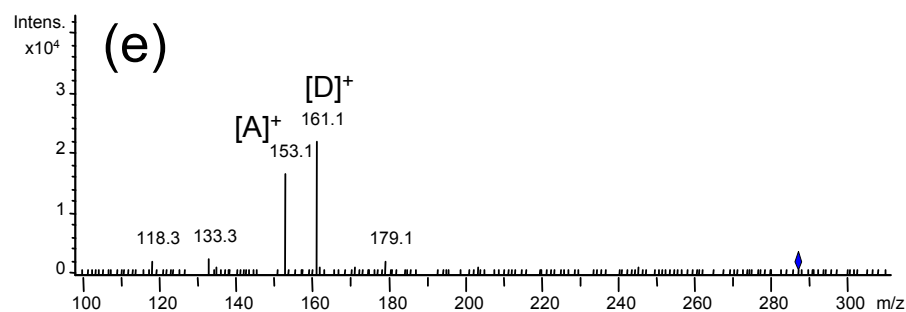
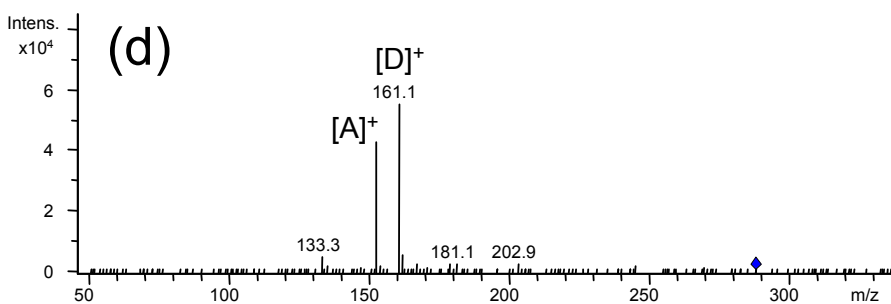
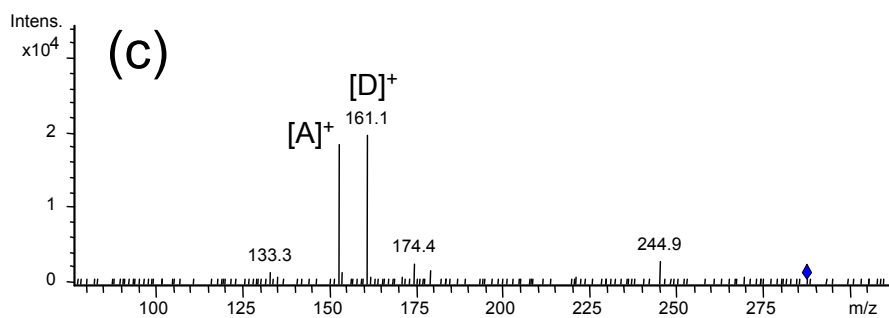
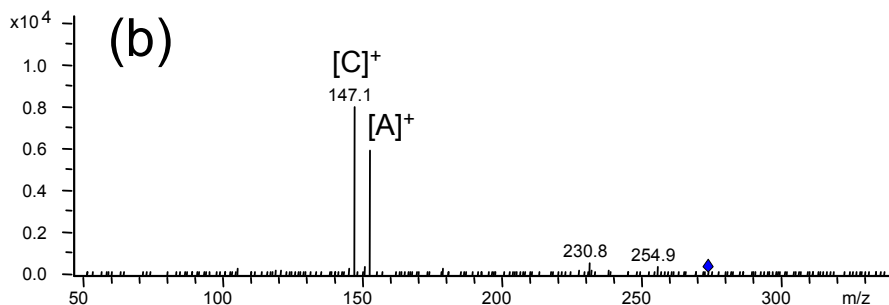
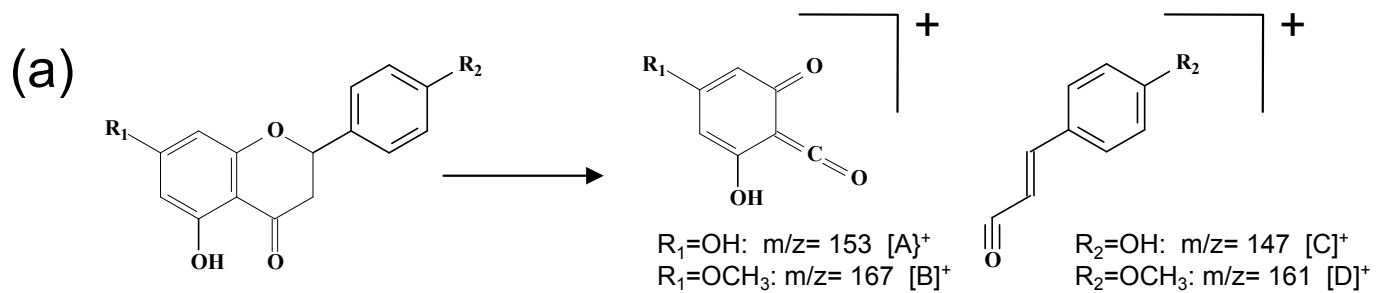


Figure S3. Positive ion LC/ESI/MSMS spectra of naringenin reaction products. (a) Proposed fragmentation scheme. (b) Naringenin, precursor ion is m/z 273. (c) MtlOMT5 methylated product, precursor ion is 287. (d) MtlOMT7 methylated product, precursor ion is 287. (e) 5,7-Dihydroxy, 4'-methoxyflavanone standard, precursor ion is 287.

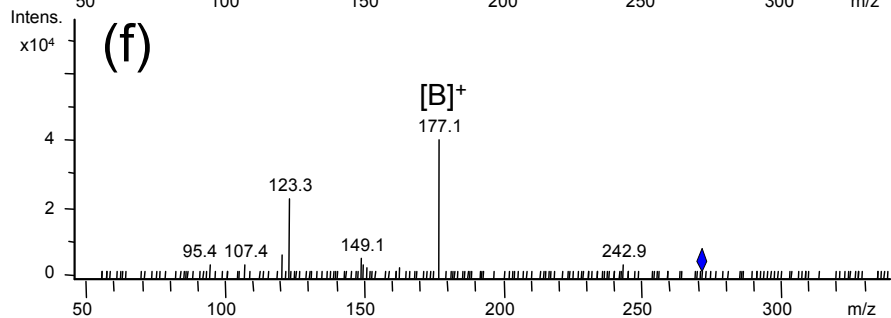
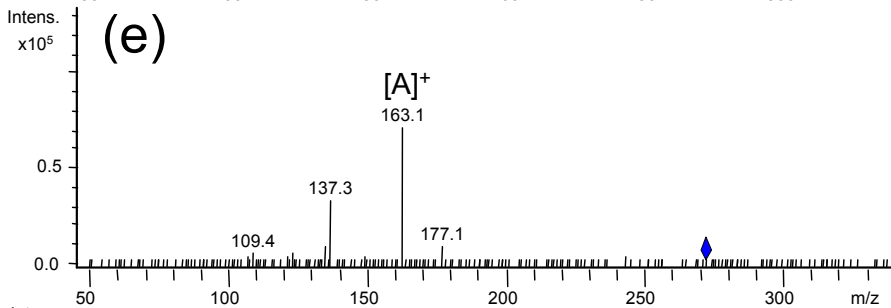
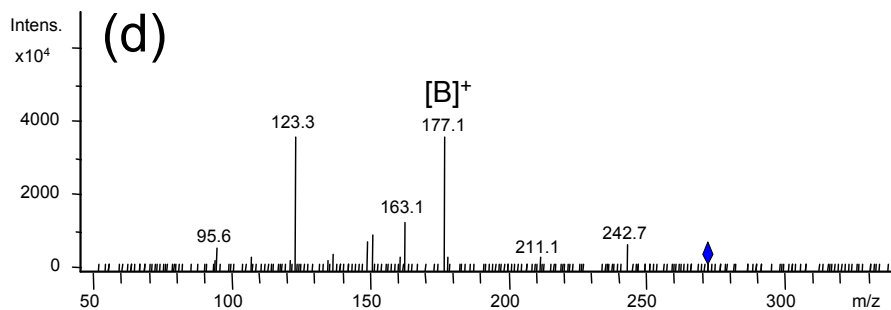
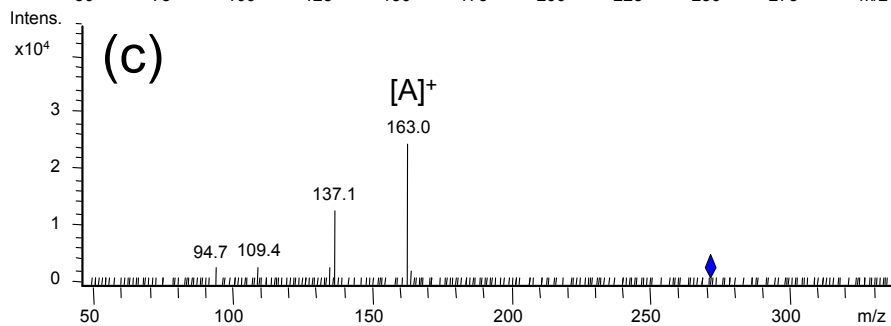
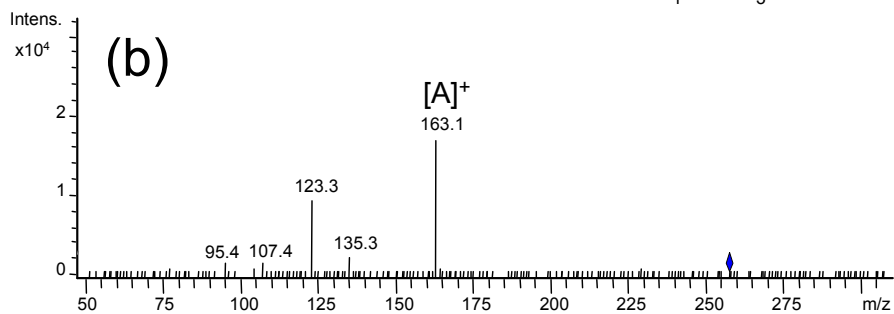
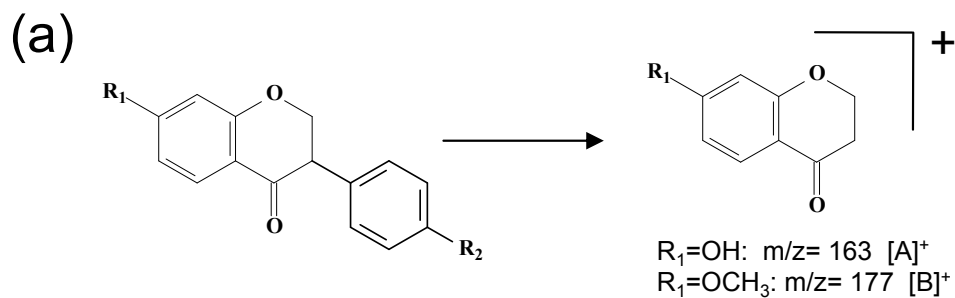


Figure S4. Positive ion LC/ESI/MSMS spectra of dihydrodaidzein reaction products. (a) Proposed fragmentation scheme. (b) Dihydrodaidzein, precursor ion is m/z 257. (c)-(d) MtlOMT5 methylated products, precursor ion is m/z 271. (e)-(f) MtlOMT7 methylated products precursor ion is m/z 271.

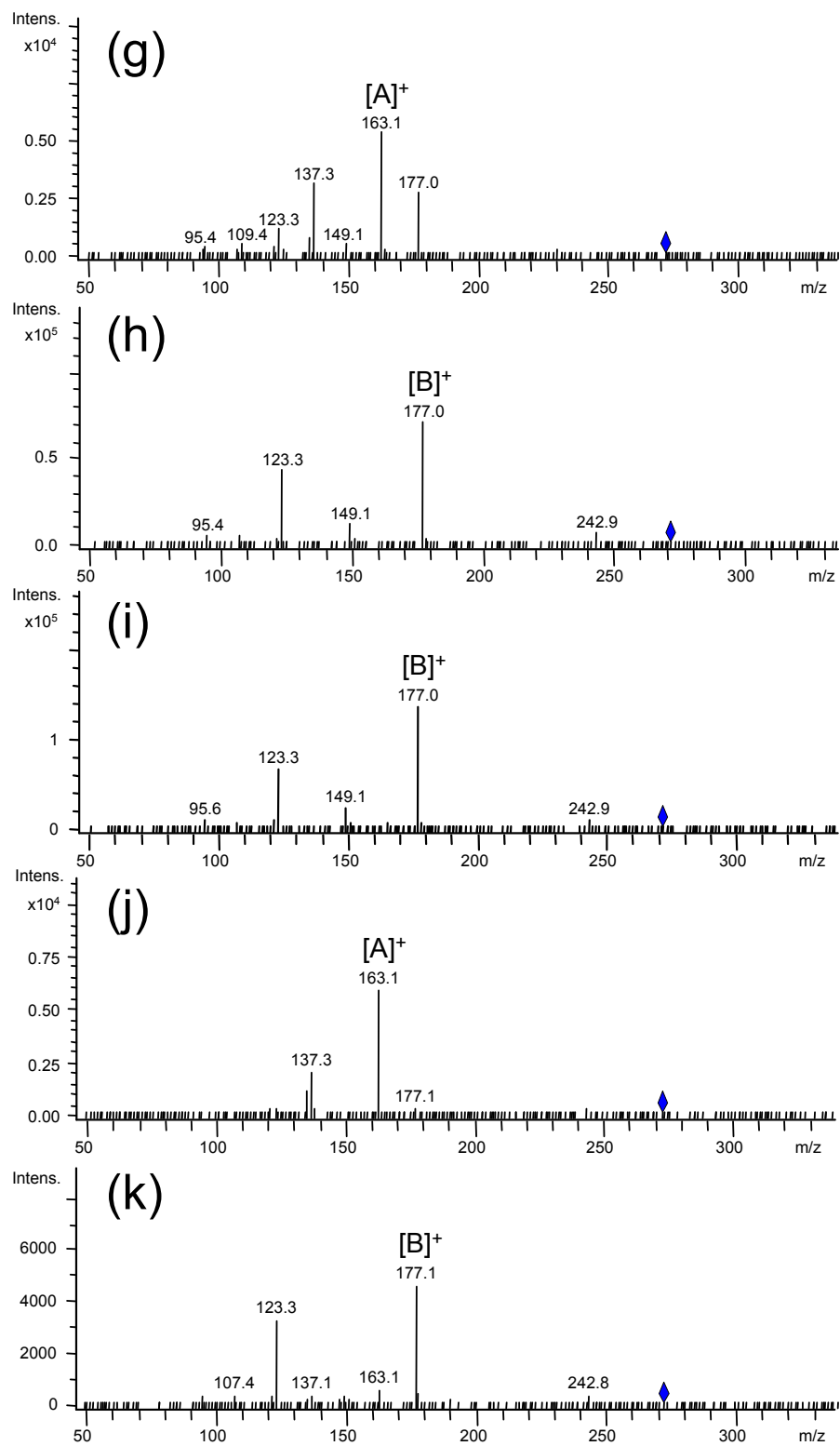


Figure S4, cont. (g)-(h) MtlOMT6 methylated products, precursor ion is m/z 271. (i) MtlOMT1 methylated product, precursor ion is m/z 271. (j)-(k) MtlOMT2 methylated products, precursor ion is m/z 271.

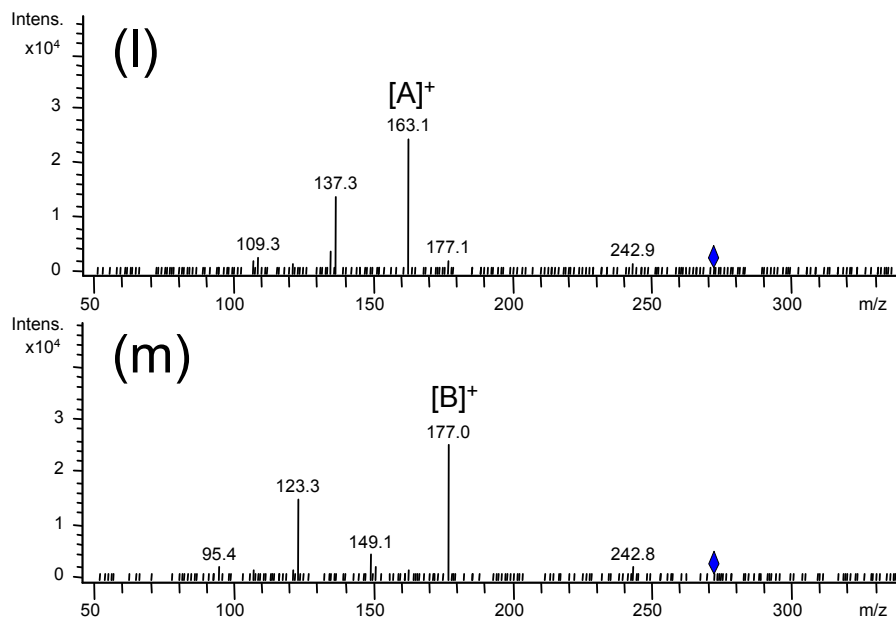


Figure S4, cont. (l)-(m). MtlOMT3 methylated products, precursor ion is m/z 271.

Table S1 Primers used for RT-PCR (listed 5' to 3')

MtIOMT	Forward primer	Reverse primer
MtIOMT1	cgggttcgtatcatgagctg	gaatgcatgatggatcaatacaaa
MtIOMT2	acaaaattttcatttgaggtgt	ggatcaaacagaaccaaacattac
MtIOMT3	gttcttgatccaacactttcaac	tccaatcatgcaaaaaccgc
MtIOMT4	ttaacaccaatgggttcttatgtctac	atacgaccgtatcaatatgcct
MtIOMT5	acaatagtgaaaggcaaagaaggagat	aactttgcagcaacttcagg
MtIOMT6	acatggaaagcctatgactgttc	acacaactccagtcccacctg
MtIOMT7	agaaacacaagacatcacccaag	cttcttctccttctttgceat

Table S2 Quantification of methylated products in reactions with dihydrodaidzein

	% 4'- Methylated	% 7- Methylated	% Dimethylated	Ratio 4'/7- methylated
MtIOMT1	0	>99	<1	0
MtIOMT2	33	66	<1	0.5
MtIOMT3	92	8	0	11.4
MtIOMT4	0	0	0	0
MtIOMT5	44	25	31	1.7
MtIOMT6	14	38	48	0.4
MtIOMT7	35	3	62	10.5