PM ROOT METHOD STUDY

P. mirifica – Summary of Root Data

A procedure for the analysis of P. mirifica root powder was tested by analyzing a triplicate set of sample 22-310-001 and a triplicate set of 22-310-001 fortified with the target analytes. Briefly, 1 gram of powdered sample is refluxed for ca. 1 hour with 100mL methanol containing 2 mL water. Following reflux, the solution is filtered and the filtrate evaporated to near dryness. The residue is taken up in methanol (three 2 to 3mL increments) to a final volume of 10.0mL. An aliquot of this solution is then analyzed by HPLC using the method developed for P. mirifica fluid extracts. HPLC analysis gave the following results for sample 22-310-001:

Analyte	concentration (mg/g)	RSD (%)
Puerarin	0.695	1.70
Daidzin	0.048	5.11
Genistin	< 0.005	NA
Daidzein	0.016	5.00
Genistein	0.005	3.44

HPLC analysis of the fortified root sample gave the following recoveries:

Analyte	recovery (%)	RSD (%)
Puerarin	NA	NA
Daidzin	98.2	0.99
Genistin	110.9*	0.65
Daidzein	96.2	0.59
Genistein	96.4	0.23

*interfering peak present.

Note 1. Manual integration employed due to baseline rise resulting from the large number of extraneous matrix components.

Note 2. Recoveries calculated on spike amounts determined from HPLC measurement of the spiking mix. Amounts spiked ranged from 150 to 250ug per target analyte. The spiking mix did not contain puerarin due to limited amounts of the standard available.

Note 3. Residual material remaining in the evaporation flask was rinsed with THF/IPA/MeCN solution. No significant target analyte amounts were found.

Note 4. In a separate experiment, a 0.5g portion of the root powder was soaked in 1.0mL ethanol overnight. A small aliquot of the ethanol was clarified with the micro-centrifuge then analyzed by HPLC. The resulting chromatogram was similar to the reflux extracts in terms of relative proportions.

root powder soaked in ethanol

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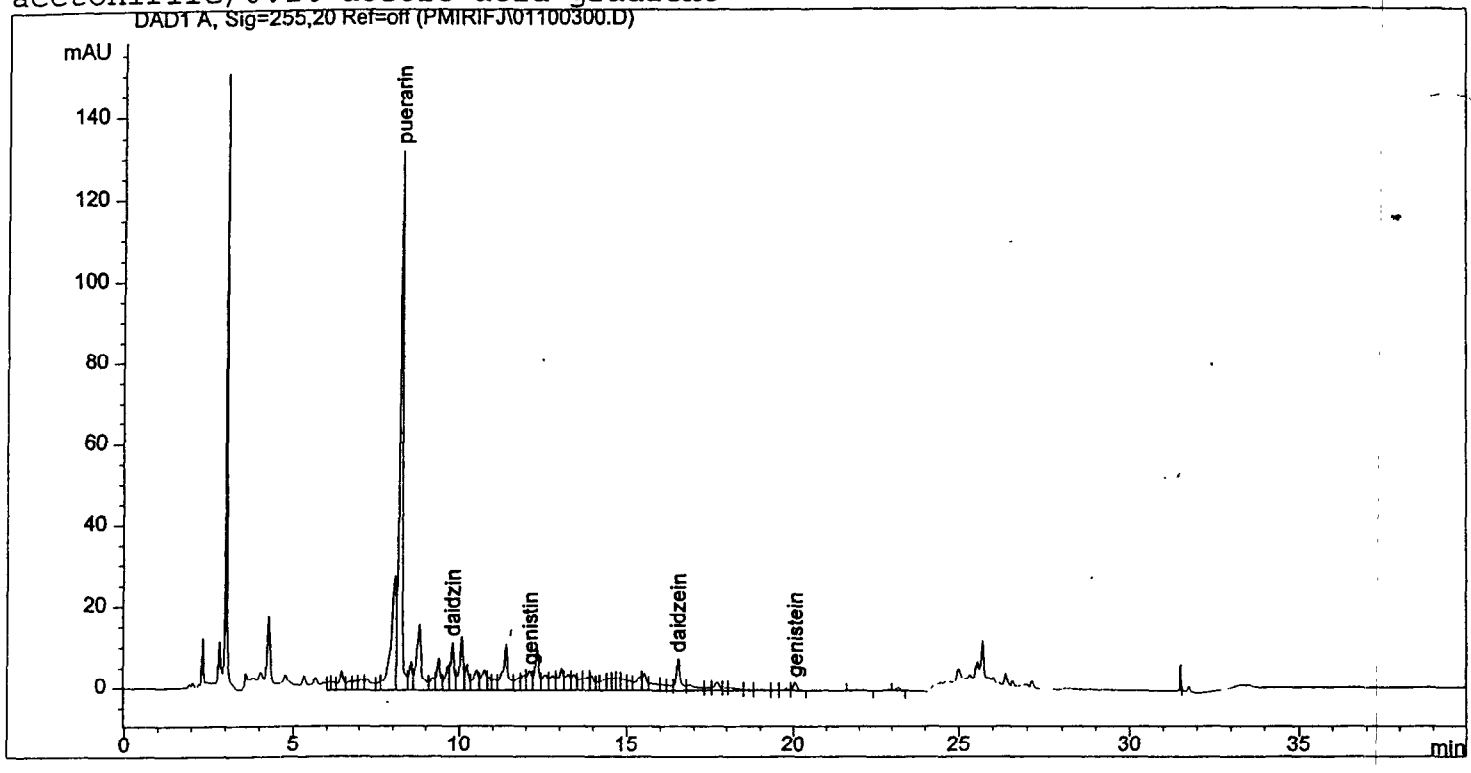
Injection Date : 1/10/03 10:42:51 AM
Sample Name : VII-180-13 Vial : 22
Acq. Operator : mr
Inj Volume : 5 µl

Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 1:25:33 PM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : Thursday, January 09, 2003 1:25:32 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.203	VV	851.73700	5.87566e-2	50.04518		puerarin
9.785	VV	80.11867	4.86630e-2	3.89881		daidzin
12.112	VV	51.39802	4.40466e-2	2.26391		genistin
16.521	VV	72.14928	3.64590e-2	2.63049		daidzein
20.059	VB	16.65584	3.20878e-2	5.34449e-1		genistein

Totals : 59.37285

Results obtained with enhanced integrator!

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*** End of Report ***

spike corrected

smith p. mirifica root Accuracy Precision, manual int.

FR Sample ID	Compound	Instrument Concentration (ug/ml)	Weight (g)	conc. ug/g
VII-179-4	puerarin	71.93045	1.0165	707.629
	daidzin	5.17552	1.0165	50.915
	genistin	0.00000	1.0165	0.000
	daidzein	1.53813	1.0165	15.132
	genistein	0.48527	1.0165	4.774
VII-179-5	puerarin	70.36590	1.0135	694.286
	daidzin	4.80463	1.0135	47.406
	genistin	0.00000	1.0135	0.000
	daidzein	1.69107	1.0135	16.685
	genistein	0.51804	1.0135	5.111
VII-179-6	puerarin	68.13508	0.9961	684.018
	daidzin	4.59907	0.9961	46.171
	genistin	0.00000	0.9961	0.000
	daidzein	1.57708	0.9961	15.833
	genistein	0.48911	0.9961	4.910
VII-179-7	puerarin	67.49717	1.0067	670.479
	daidzin	20.43260	1.0067	202.966
	genistin	27.43559	1.0067	272.530
	daidzein	22.64884	1.0067	224.981
	genistein	19.74738	1.0067	196.160
VII-179-8	puerarin	69.30040	1.0236	677.026
	daidzin	20.67603	1.0236	201.993
	genistin	27.53441	1.0236	268.996
	daidzein	22.48235	1.0236	219.640
	genistein	19.83376	1.0236	193.765
VII-179-9	puerarin	64.83416	0.9999	648.406
	daidzin	20.80716	0.9999	208.092
	genistin	27.18898	0.9999	271.917
	daidzein	22.38444	0.9999	223.867
	genistein	19.82429	0.9999	198.263

VOLUME IN ML
10

dilution factor
1

		average	std. dev.	RSD	RPD vs. auto int.
precision	puerarin	695.311	11.838	1.703	.6%
	daidzin	48.164	2.461	5.110	8.2%
	genistin	0.000	0.000	#DIV/0!	
	daidzein	15.883	0.778	4.899	28.7%
	genistein	4.932	0.170	3.442	5.5%

VII-179-7		spike recoveries			
Analyte	**sample mean**	**sample wt.**	**amount**	**spike**	**total**
puerarin	695.311	1.0067	699.97	0.0	700.0
daidzin	48.164	1.0067	48.49	161.5	210.0
genistin	0	1.0067	0.00	247.0	247.0
daidzein	15.883	1.0067	15.99	218.0	234.0
genistein	4.932	1.0067	4.97	200.5	205.5
Analyte	**found**	**sample wt.**	**amount**	**recovery**	
puerarin	670.479	1.0067	674.97	96.4	
daidzin	202.966	1.0067	204.33	97.3	
genistin	272.53	1.0067	274.36	111.1	
daidzein	224.981	1.0067	226.49	96.8	
genistein	196.16	1.0067	197.47	96.1	

VII-179-8		spike recoveries			
Analyte	**sample mean**	**sample wt.**	**amount**	**spike**	**total**
puerarin	695.311	1.0236	711.72	0.0	711.7
daidzin	48.164	1.0236	49.30	161.5	210.8
genistin	0	1.0236	0.00	247.0	247.0
daidzein	15.883	1.0236	16.26	218.0	234.3
genistein	4.932	1.0236	5.05	200.5	205.5
Analyte	**found**	**sample wt.**	**amount**	**recovery**	
puerarin	677.026	1.0236	693.00	97.4	
daidzin	201.993	1.0236	206.76	98.1	
genistin	268.996	1.0236	275.34	111.5	
daidzein	219.64	1.0236	224.82	96.0	
genistein	193.765	1.0236	198.34	96.5	

VII-179-9		spike recoveries			
Analyte	**sample mean**	**sample wt.**	**amount**	**spike**	**total**
puerarin	695.311	0.9999	695.24	0.0	695.2
daidzin	48.164	0.9999	48.16	161.5	209.7
genistin	0	0.9999	0.00	247.0	247.0
daidzein	15.883	0.9999	15.88	218.0	233.9
genistein	4.932	0.9999	4.93	200.5	205.4
Analyte	**found**	**sample wt.**	**amount**	**recovery**	
puerarin	648.406	0.9999	648.34	93.3	
daidzin	208.092	0.9999	208.07	99.2	
genistin	271.917	0.9999	271.89	110.1	
daidzein	223.867	0.9999	223.84	95.7	
genistein	198.263	0.9999	198.24	96.5	

		average	std. dev.		RSD
accuracy	puerarin	95.684	2.157		2.254
	daidzin	98.210	0.975		0.993
	genistin	110.876	0.720		0.650
	daidzein	96.159	0.566		0.589
	genistein	96.368	0.223		0.231

:a File C:\HPCHEM\1\DATA\PMIRIFJ\01080312.D Sample Name: VII-179-9

spike rep 3

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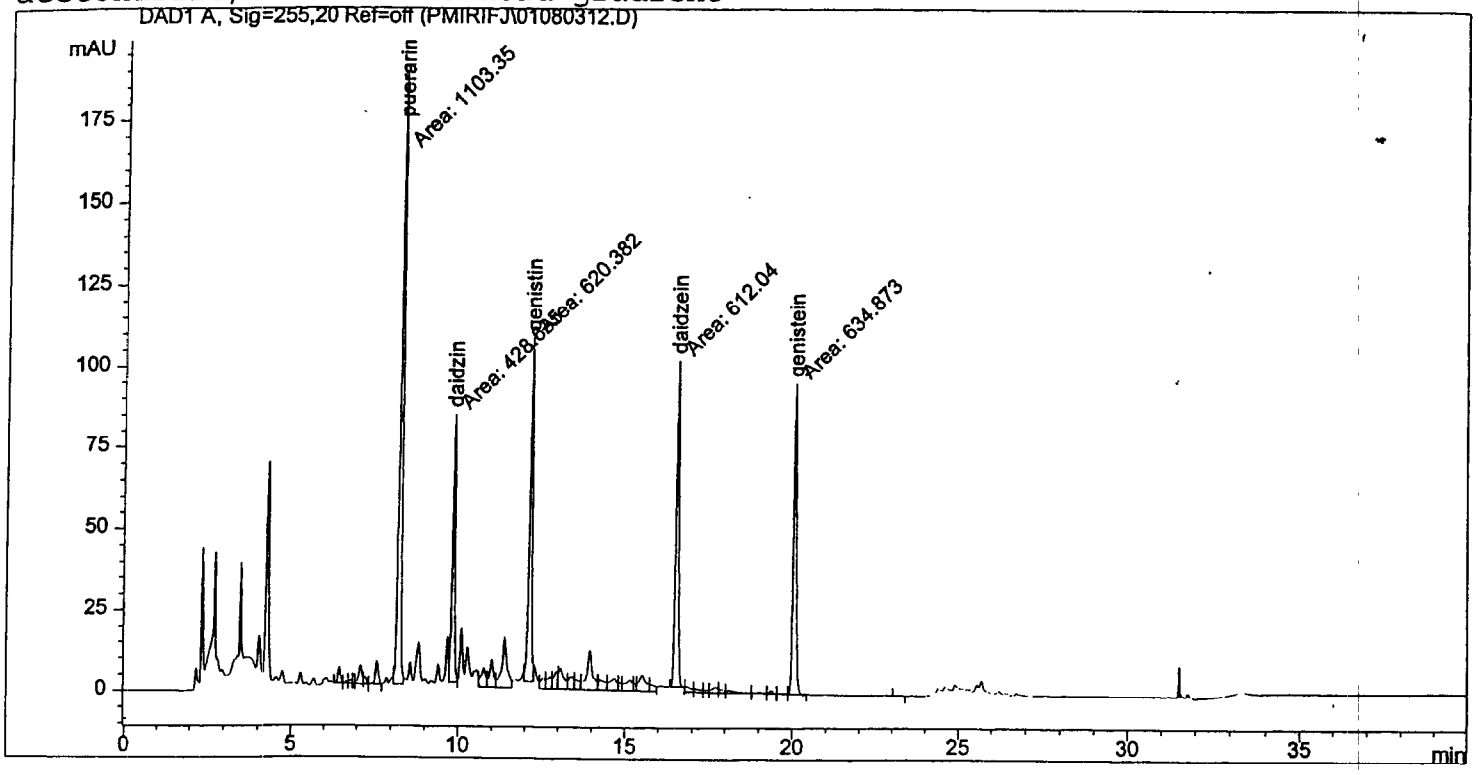
Injection Date : 1/8/03 10:06:26 PM Seq. Line : 12
Sample Name : VII-179-9 Vial : 12
Acq. Operator : mr Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 1:25:33 PM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.212	MF	1103.35046	5.87612e-2	64.83416		puerarin
9.809	MF	428.63550	4.85428e-2	20.80716		daidzin
12.114	MF	620.38165	4.38262e-2	27.18898		genistin
16.505	MM	612.03973	3.65735e-2	22.38444		daidzein
20.038	MF	634.87292	3.12256e-2	19.82429		genistein

no page 2 added

sample 22-310-001 rep1

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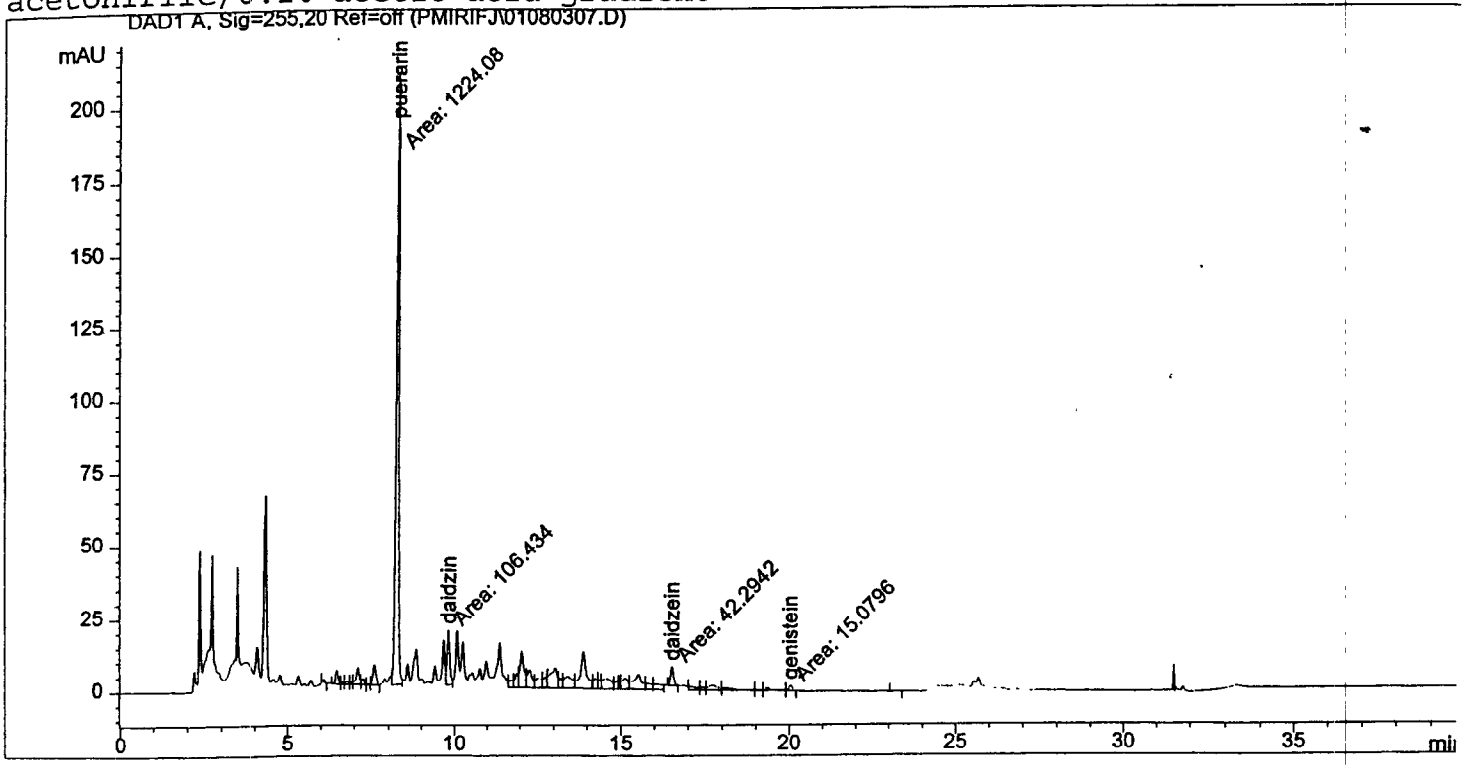
Injection Date : 1/8/03 6:40:47 PM
Sample Name : VII-179-4
Acq. Operator : mr

Seq. Line : 7
Vial : 7
Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 1:25:33 PM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : Thursday, January 09, 2003 1:25:32 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.226	MF	1224.08386	5.87627e-2	71.93045		puerarin
9.803	MF	106.43434	4.86264e-2	5.17552		daidzin
12.124		-	-	-		genistin
16.506	MF	42.29419	3.63673e-2	1.53813		daidzein
20.040	MF	15.07957	3.21803e-2	4.85265e-1		genistein

:a File C:\HPCHEM\1\DATA\PMIRIFJ\01080308.D Sample Name: VII-179-5

rep 2

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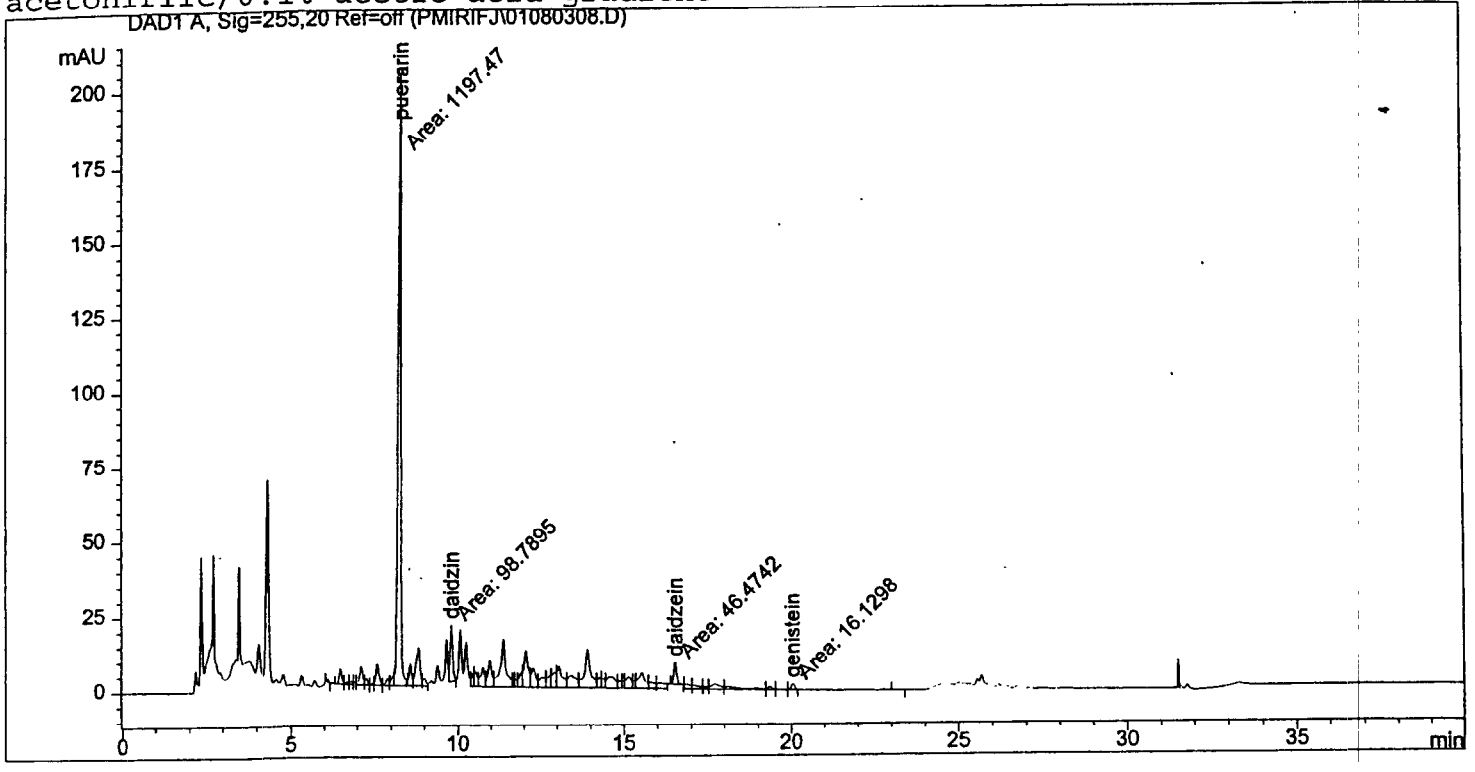
Injection Date : 1/8/03 7:21:54 PM
Sample Name : VII-179-5
Acq. Operator : mr

Seq. Line : 8
Vial : 8
Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 1:25:33 PM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : Thursday, January 09, 2003 1:25:32 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.227	MF	1197.46521	5.87624e-2	70.36590		puerarin
9.800	MF	98.78951	4.86351e-2	4.80463		daidzin
12.124		-	-	-		genistin
16.512	MM	46.47417	3.63873e-2	1.69107		daidzein
20.038	MF	16.12980	3.21166e-2	5.18035e-1		genistein

no page of, end of report

a File C:\HPCHEM\1\DATA\PMIRIFJ\01080309.D Sample Name: VII-179-6

rep 3

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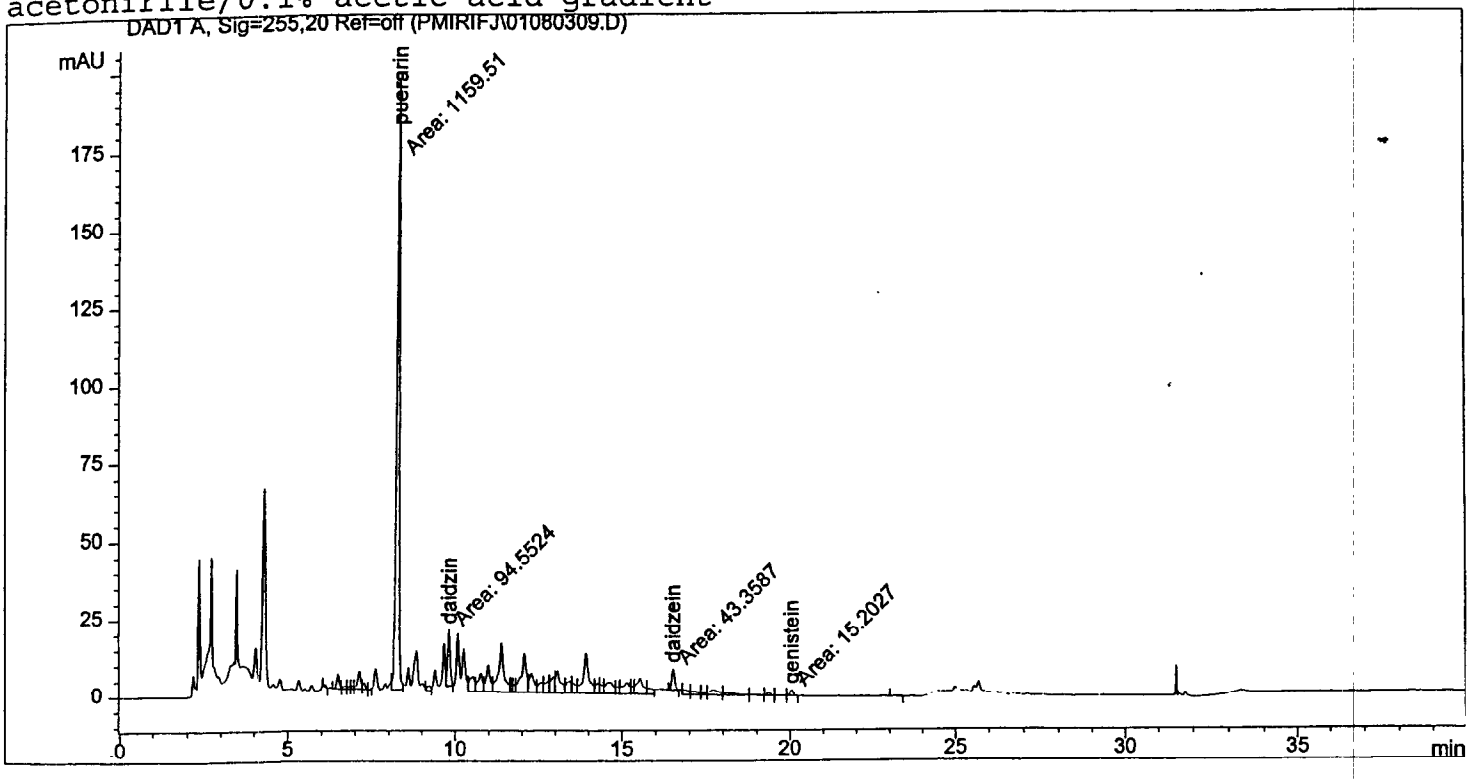
Injection Date : 1/8/03 8:03:00 PM
Sample Name : VII-179-6
Acq. Operator : mr

Seq. Line : 9
Vial : 9
Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 1:25:33 PM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
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Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.233	MF	1159.51099	5.87619e-2	68.13508		puerarin
9.805	MF	94.55237	4.86404e-2	4.59907		daidzin
12.124		-	-	-		genistin
16.510	MF	43.35871	3.63728e-2	1.57708		daidzein
20.040	MF	15.20274	3.21724e-2	4.89108e-1		genistein

no page 2. end of report

.a File C:\HPCHEM\1\DATA\PMIRIFJ\01080310.D Sample Name: VII-179-7

spike rep 1

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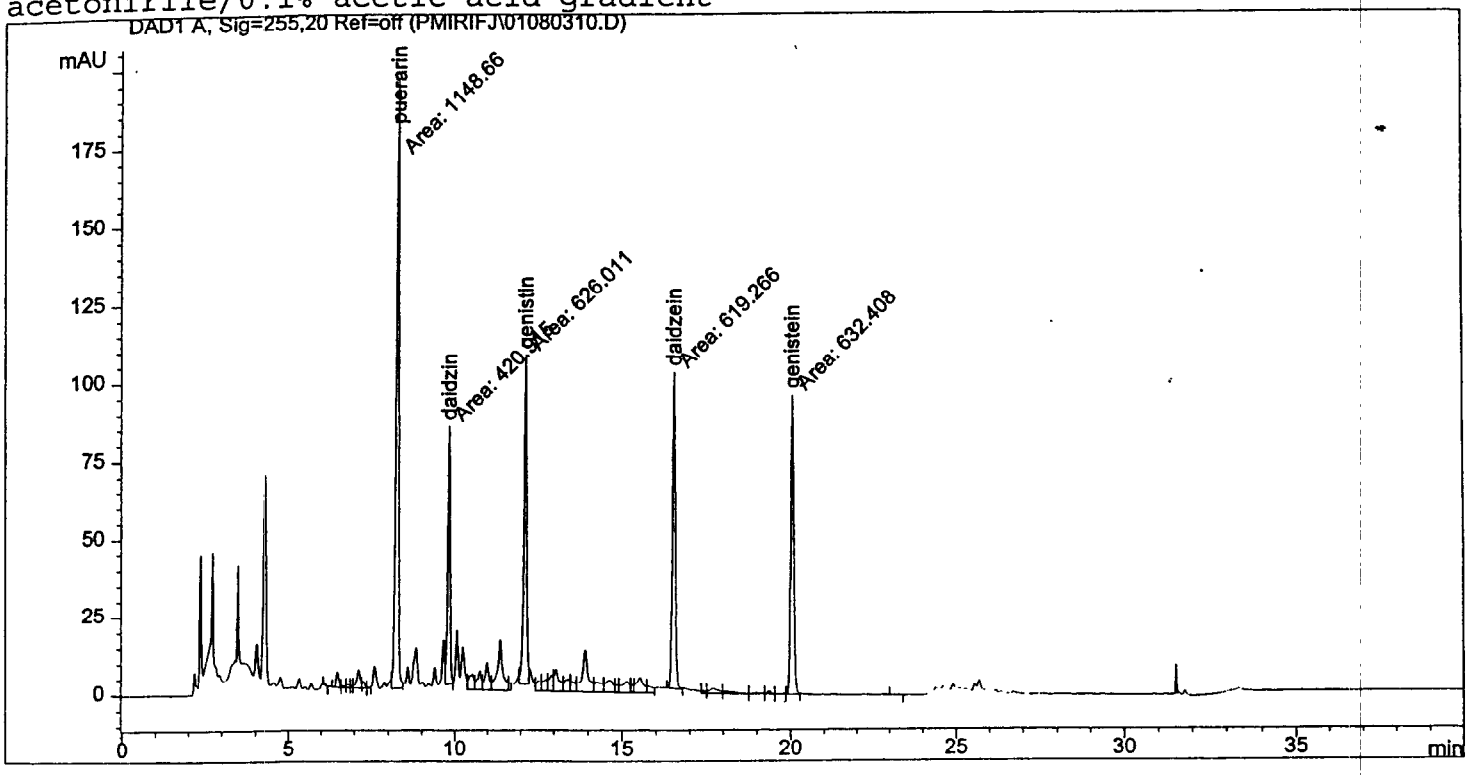
Injection Date : 1/8/03 8:44:11 PM
Sample Name : VII-179-7
Acq. Operator : mr

Seq. Line : 10
Vial : 10
Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 1:25:33 PM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : Thursday, January 09, 2003 1:25:32 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.232	FM	1148.65784	5.87618e-2	67.49717		puerarin
9.806	MF	420.91495	4.85433e-2	20.43260		daidzin
12.118	MF	626.01129	4.38260e-2	27.43559		genistin
16.505	MF	619.26617	3.65737e-2	22.64884		daidzein
20.036	MF	632.40802	3.12257e-2	19.74738		genistein

no page 2 end of report

spike rep 2

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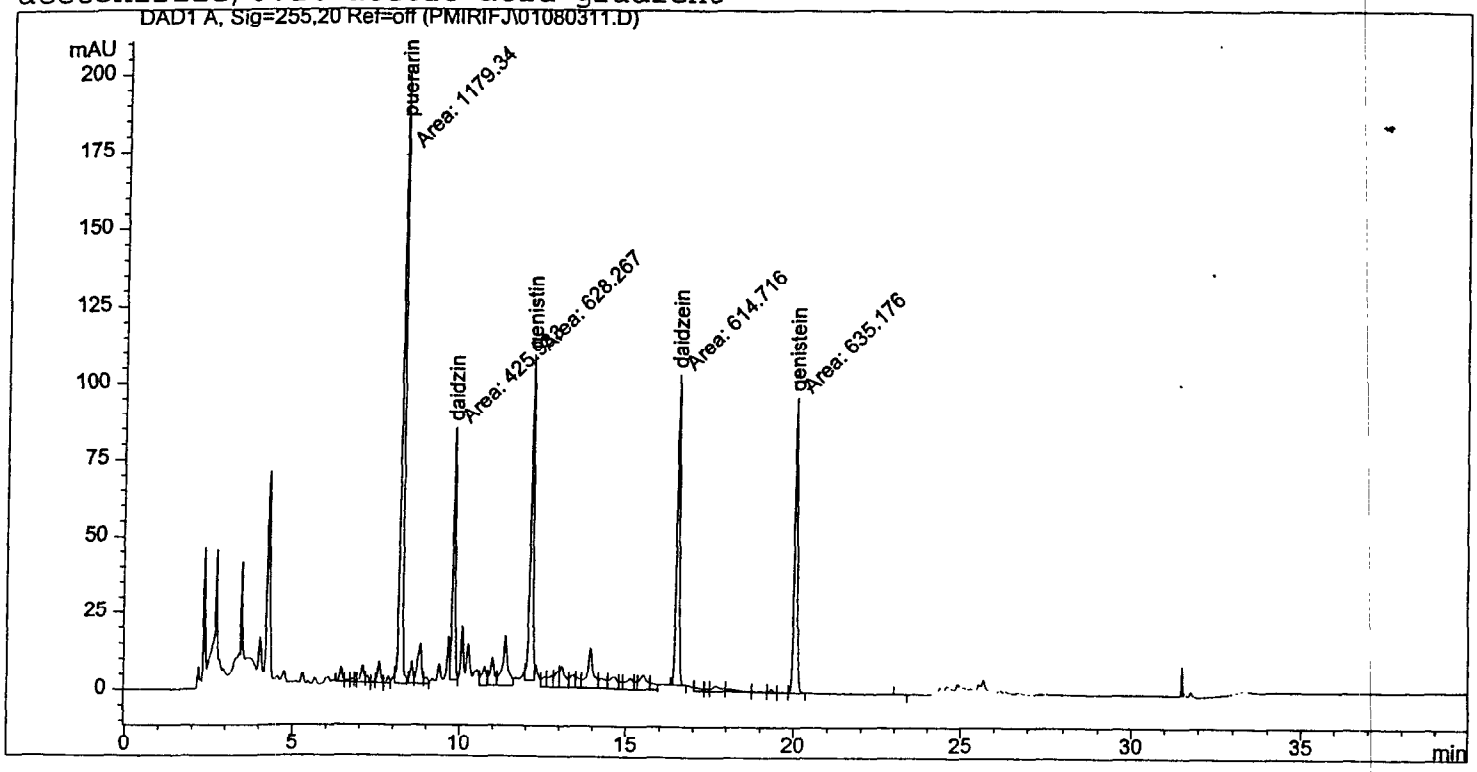
Injection Date : 1/8/03 9:25:19 PM Seq. Line : 11
Sample Name : VII-179-8 Vial : 11
Acq. Operator : mr Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 1:25:33 PM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : Thursday, January 09, 2003 1:25:32 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.207	MF	1179.33728	5.87622e-2	69.30040		puerarin
9.803	MF	425.93259	4.85430e-2	20.67603		daidzin
12.116	MF	628.26703	4.38260e-2	27.53441		genistin
16.502	MM	614.71582	3.65736e-2	22.48235		daidzein
20.030	MF	635.17639	3.12256e-2	19.83376		genistein

:a File C:\HPCHEM\1\DATA\PMIRIFJ\01080314.D Sample Name: stdIV-26-9

spike mix

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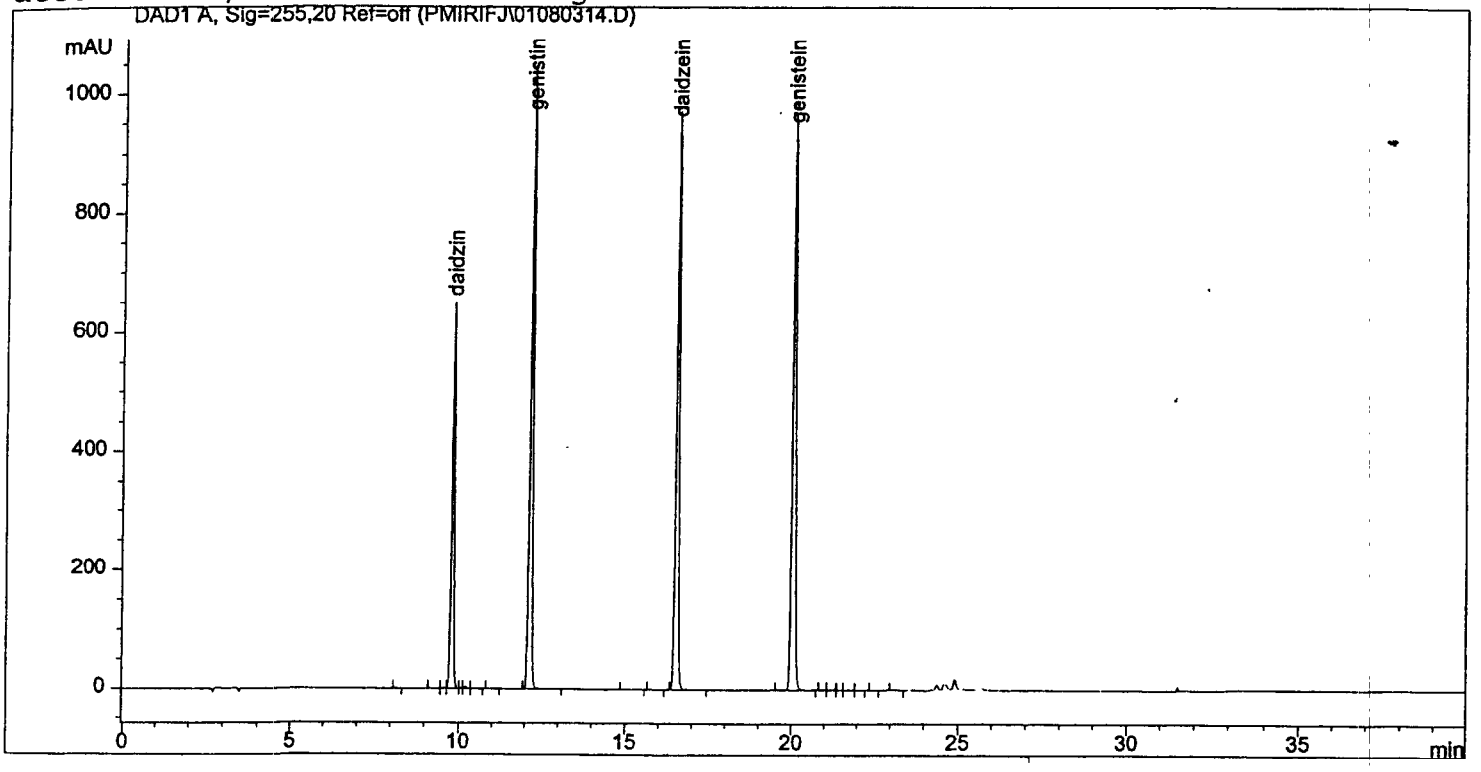
Injection Date : 1/8/03 11:28:39 PM Seq. Line : 14
Sample Name : stdIV-26-9 Vial : 13
Acq. Operator : mr Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 10:37:43 AM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/9/03 10:27:38 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.239		-	-	-		puerarin
9.783	VV	3329.29443	4.85187e-2	161.53309		daidzin
12.132	BB	5639.08691	4.38085e-2	247.03991		genistin
16.507	BB	5967.85400	3.65872e-2	218.34729		daidzein
20.030	VB	6424.32129	3.12047e-2	200.46884		genistein

no page 2, end of report

1/7/03 P. mirifica - Root

FR # 22-310-001

	sample	weight	spike STD IV-26-9
	VII-179-4	1.0165 g	—
	VII-179-5	1.0135 g	—
	VII-179-6	0.9961 g	—
	VII-179-7	1.0067 g	1.0 mL
	VII-179-8	1.0236 g	1.0 mL
	VII-179-9	0.9999 g	1.0 mL
Method blank	VII-179-10	—	—

sample transfered to 250 mL round bottom.
Add 2 mL H_2O and 100 mL MeOH (#22-287-001).
reflux ca. 1 hour on steam table.
stand overnight. MR

1/8/03 filter through coarse paper grade 313 (VWR #28333-043) into 500 mL round bottoms. Rinse 3 x (2-5 mL) MeOH + add rinsings to filter. Rinse filter w/ MeOH ca. 2-3 mL.
Roto-vap to near dryness @ 45 °C.
Residue taken up 3x 2 to 3 mL MeOH/H_2O (8:2) SOL II-194-7, each rinsing transfered to 10 mL volumetric.
mixed well after bring up to volume

note: noticable insoluble residue remains in flask + transfered to volumetric.

filtered aliquot of sample solution for analysis using syringe ([illegible] lot 00B0284) and PTFE filter 45 [illegible] (Whatman #6784-1304) MR

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Calibration Table

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Calib. Data Modified : 1/9/03 10:27:38 AM

Calculate : External Standard
Based on : Peak Area

Rel. Reference Window : 0.800 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 0.800 %
Abs. Non-ref. Window : 0.000 min
Uncalibrated Peaks : not reported
Partial Calibration : Yes, identified peaks are recalibrated
Correct All Ret. Times: No, only for identified peaks

Curve Type : Linear
Origin : Ignored
Weight : Linear (Amnt)

Recalibration Settings:
Average Response : Average all calibrations
Average Retention Time: Floating Average New 75%

Calibration Report Options :
Printout of recalibrations within a sequence:
Calibration Table after Recalibration
Normal Report after Recalibration
If the sequence is done with bracketing:
Results of first cycle (ending previous bracket)

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Sig	Lvl	Amount [ug/mL]	Area	Amt/Area	Ref	Grp	Name
8.239	1	1	4.26000e-1	7.61312	5.59560e-2			puerarin
		2	4.26000	71.75446	5.93691e-2			
		3	42.60000	729.09467	5.84286e-2			
		4	85.10000	1445.05408	5.88905e-2			
9.808	1	1	4.53000e-1	9.21783	4.91439e-2			daidzin
		2	4.53000	91.64769	4.94284e-2			
		3	45.30000	936.81451	4.83554e-2			
		4	90.60000	1865.23999	4.85728e-2			
12.124	1	1	5.58000e-1	12.55510	4.44441e-2			genistin
		2	5.58000	125.74725	4.43747e-2			
		3	55.80000	1279.41846	4.36136e-2			
		4	111.60000	2542.63599	4.38915e-2			
16.516	1	1	5.45000e-1	15.31533	3.55853e-2			daidzein
		2	5.45000	147.20198	3.70240e-2			
		3	54.50000	1495.25903	3.64485e-2			
		4	109.00000	2975.67529	3.66303e-2			
20.046	1	1	5.05000e-1	15.94846	3.16645e-2			genistein
		2	5.05000	158.78447	3.18041e-2			
		3	50.50000	1620.15015	3.11700e-2			
		4	101.00000	3236.65723	3.12050e-2			

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Peak Sum Table

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No Entries in table

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Calibration Curves

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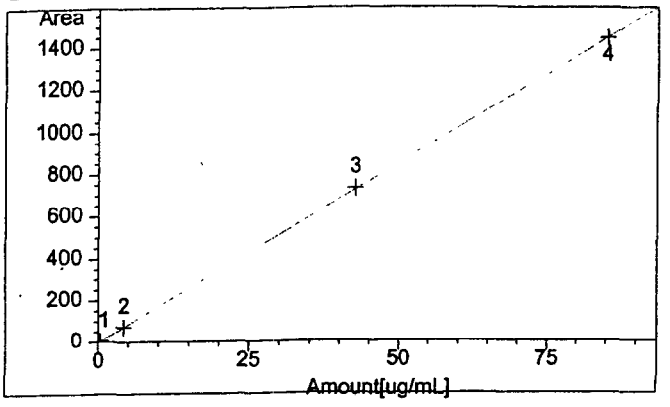
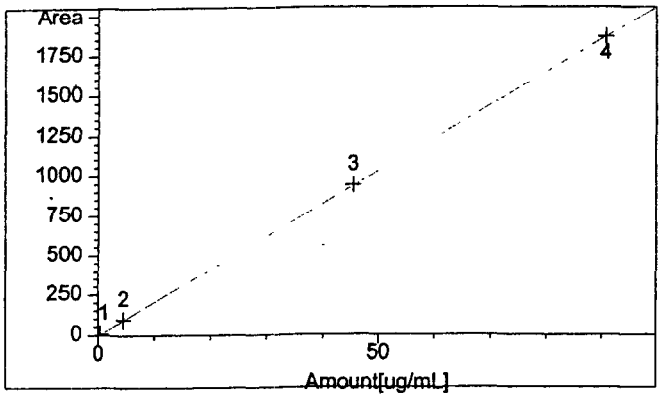
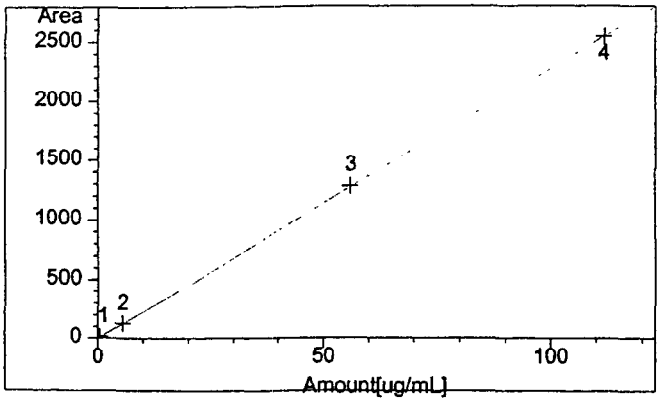


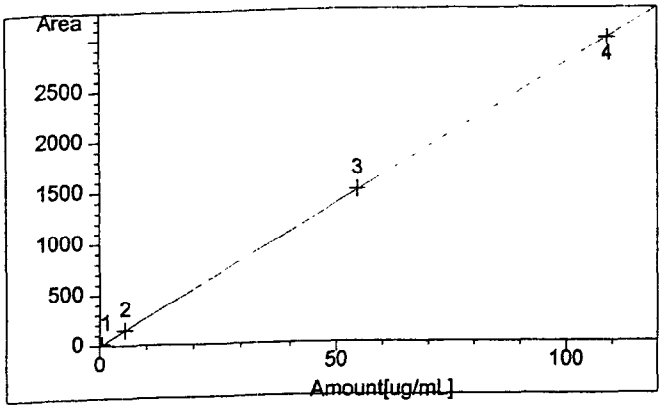
puerarin at exp. RT: 8.239
DAD1 A, Sig=255,20 Ref=off
Correlation: 0.99999
Residual Std. Dev.: 3.66072
Formula: y = mx + b
m: 17.01359
b: 2.88899e-1
x: Amount [ug/mL]
y: Area
Calibration Level Weights:
Level 1 : 1
Level 2 : 0.1
Level 3 : 0.01
Level 4 : 0.005006



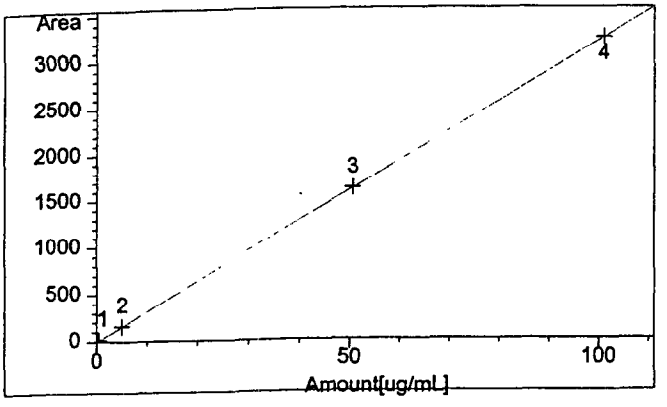
daidzin at exp. RT: 9.808
DAD1 A, Sig=255,20 Ref=off
Correlation: 0.99999
Residual Std. Dev.: 2.93186
Formula: y = mx + b
m: 20.61211
b: -2.44132e-1
x: Amount [ug/mL]
y: Area
Calibration Level Weights:
Level 1 : 1
Level 2 : 0.1
Level 3 : 0.01
Level 4 : 0.005



genistin at exp. RT: 12.124
DAD1 A, Sig=255,20 Ref=off
Correlation: 0.99999
Residual Std. Dev.: 5.40807
Formula: y = mx + b
m: 22.82776
b: -2.81960e-1
x: Amount [ug/mL]
y: Area
Calibration Level Weights:
Level 1 : 1
Level 2 : 0.1
Level 3 : 0.01
Level 4 : 0.005



daidzein at exp. RT: 16.516
DAD1 A, Sig=255,20 Ref=off
Correlation: 0.99999
Residual Std. Dev.: 4.86057
Formula: y = mx + b
m: 27.33076
b: 2.55993e-1
x: Amount [ug/mL]
y: Area
Calibration Level Weights:
Level 1 : 1
Level 2 : 0.1
Level 3 : 0.01
Level 4 : 0.005



genistein at exp. RT: 20.046
DAD1 A, Sig=255,20 Ref=off
Correlation: 1.00000
Residual Std. Dev.: 2.39270
Formula: y = mx + b
m: 32.04884
b: -4.72617e-1
x: Amount [ug/mL]
y: Area
Calibration Level Weights:
Level 1 : 1
Level 2 : 0.1
Level 3 : 0.01
Level 4 : 0.005

==========

.a File C:\HPCHEM\1\DATA\PMIRIFJ\01080302.D Sample Name: stdIV-12-19

cal std. @ ca. 0.5 ug/mL

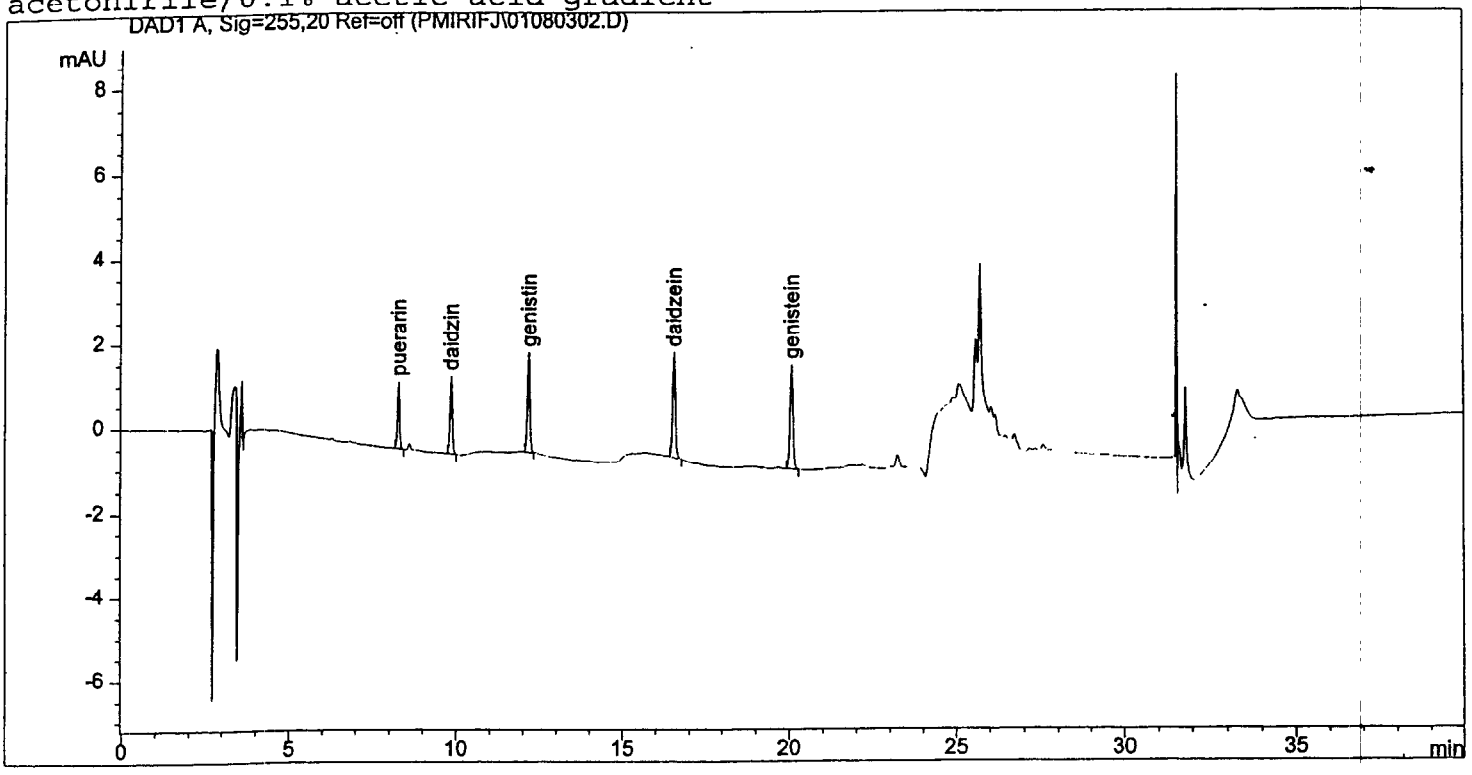
==========

Injection Date : 1/8/03 3:15:12 PM
Sample Name : stdIV-12-19
Acq. Operator : mr

Seq. Line : 2
Vial : 2
Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 10:26:18 AM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : Thursday, January 09, 2003 10:25:51 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name	
8.236	BP	7.61312	5.65461e-2	4.30493e-1		puerarin	101.0 %
9.800	BB	9.21783	4.98001e-2	4.59049e-1		daidzin	101.3 %
12.119	BB	12.55510	4.47901e-2	5.62344e-1		genistin	100.8 %
16.512	BB	15.31533	3.59772e-2	5.51003e-1		daidzein	101.1 %
20.044	BB	15.94846	3.21270e-2	5.12377e-1		genistein	101.5 %

no page 2, end of report

a File C:\HPCHEM\1\DATA\PMIRIFJ\01080303.D Sample Name: stdIV-12-10

cal. std. @ ca. 5 ug/mL

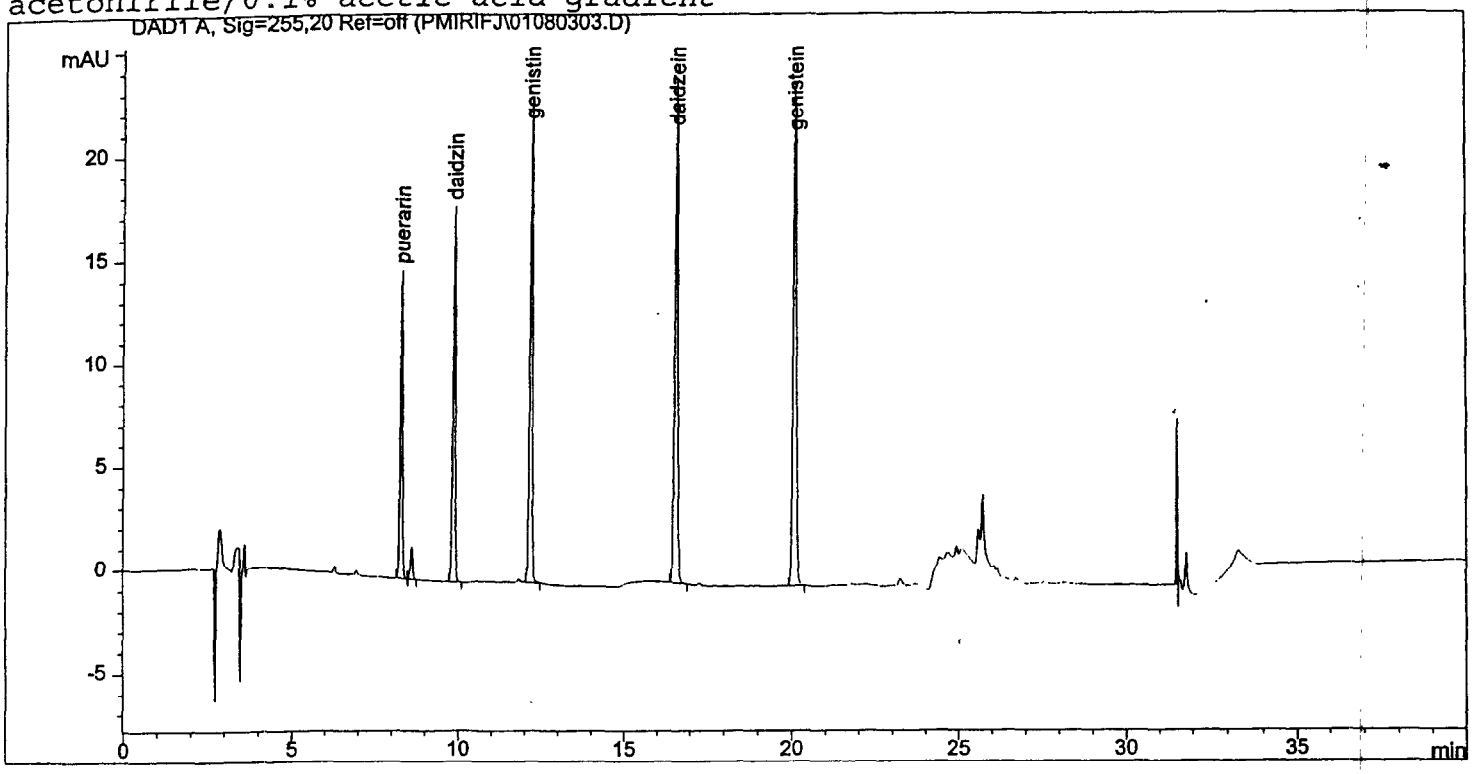
==========

Injection Date : 1/8/03 3:56:17 PM
Sample Name : stdIV-12-10
Acq. Operator : mr

Seq. Line : 3
Vial : 3
Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 10:27:48 AM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/9/03 10:27:38 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name	
8.232	BV	71.75446	5.85399e-2	4.20050		puerarin	98.6%
9.803	BB	91.64769	4.86444e-2	4.45815		daidzin	98.4%
12.126	BB	125.74725	4.39045e-2	5.52087		genistin	98.9%
16.516	BB	147.20198	3.65252e-2	5.37658		daidzein	98.6%
20.046	BB	158.78447	3.12952e-2	4.96920		genistein	98.4%

no page 2, end of report

a File C:\HPCHEM\1\DATA\PMIRIFJ\01080304.D Sample Name: stdIV-12-1

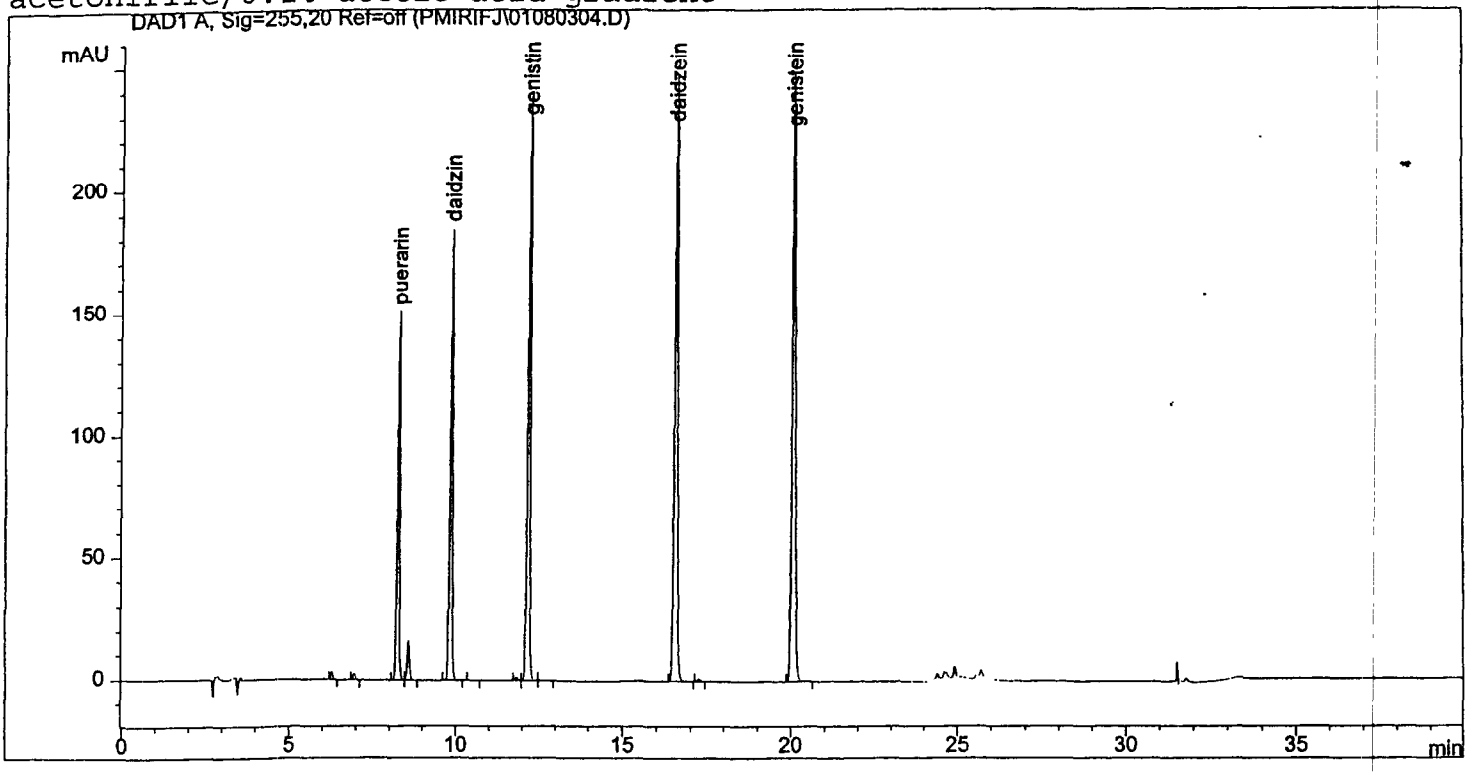
cal. std. @ ca. 50 ug/mL

==========

Injection Date : 1/8/03 4:37:25 PM Seq. Line : 4
Sample Name : stdIV-12-1 Vial : 4
Acq. Operator : mr Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 10:27:48 AM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/9/03 10:27:38 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name	
8.237	VV	729.09467	5.87533e-2	42.83669		puerarin	100.6%
9.808	BB	936.81451	4.85278e-2	45.46155		daidzin	100.4%
12.127	VV	1279.41846	4.38160e-2	56.05895		genistin	100.5%
16.519	BB	1495.25903	3.65825e-2	54.70038		daidzein	100.4%
20.049	PB	1620.15015	3.12115e-2	50.56728		genistein	100.1%

no page 2, end of report

a File C:\HPCHEM\1\DATA\PMIRIFJ\01080305.D Sample Name: stdIII-200-1

cal. std. @ ca. 100 ug/mL

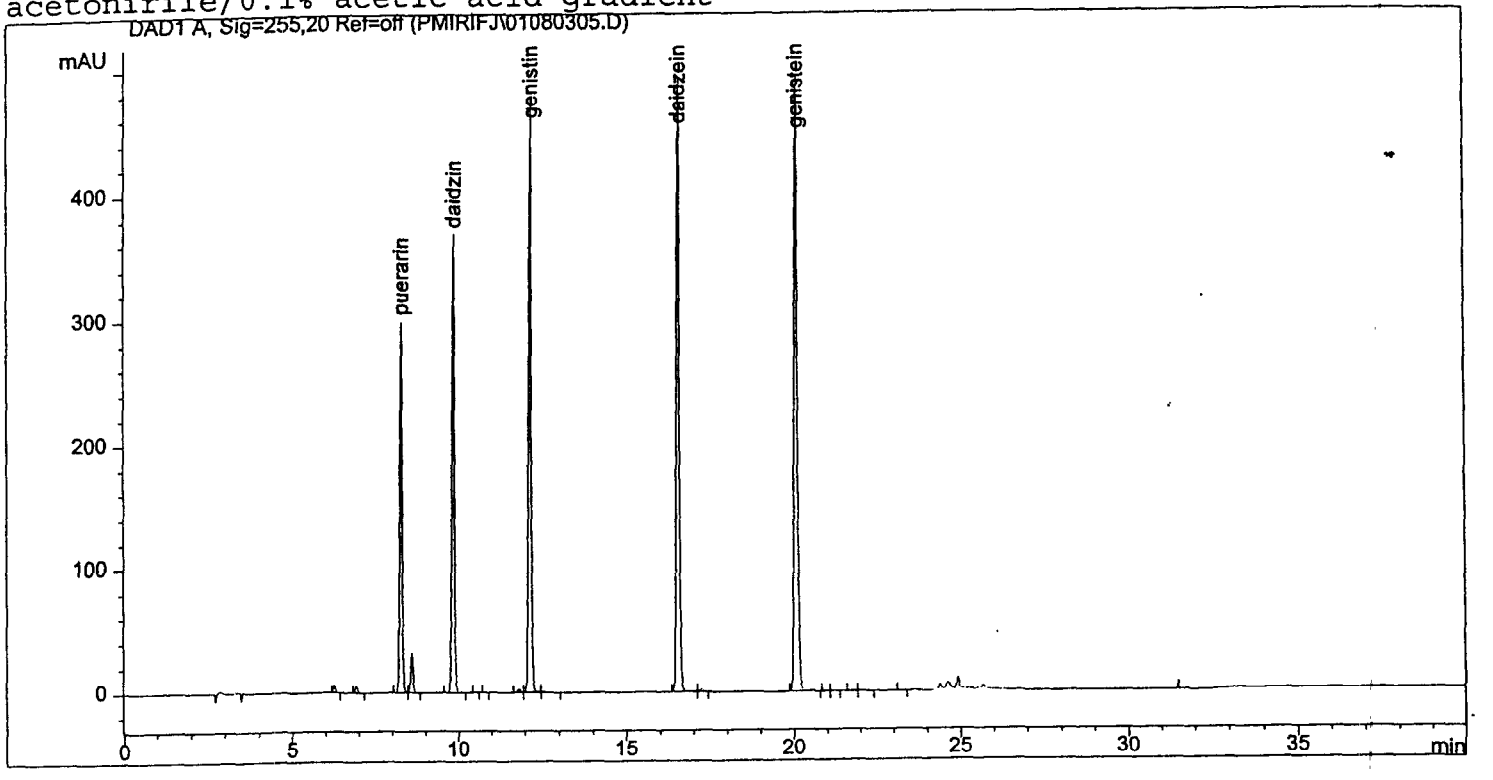
==========

Injection Date : 1/8/03 5:18:33 PM
Sample Name : stdIII-200-1
Acq. Operator : mr

Seq. Line : 5
Vial : 5
Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 10:27:48 AM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/9/03 10:27:38 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name	
8.250	VV	1445.05408	5.87648e-2	84.91832		puerarin	99.8%
9.814	VB	1865.23999	4.85215e-2	90.50426		daidzin	99.9%
12.129	VV	2542.63599	4.38112e-2	111.39584		genistin	99.8%
16.524	BB	2975.67529	3.65857e-2	108.86704		daidzein	99.9%
20.055	BB	3236.65723	3.12069e-2	101.00614		genistein	100.0%

no page 2, end of report

method blank

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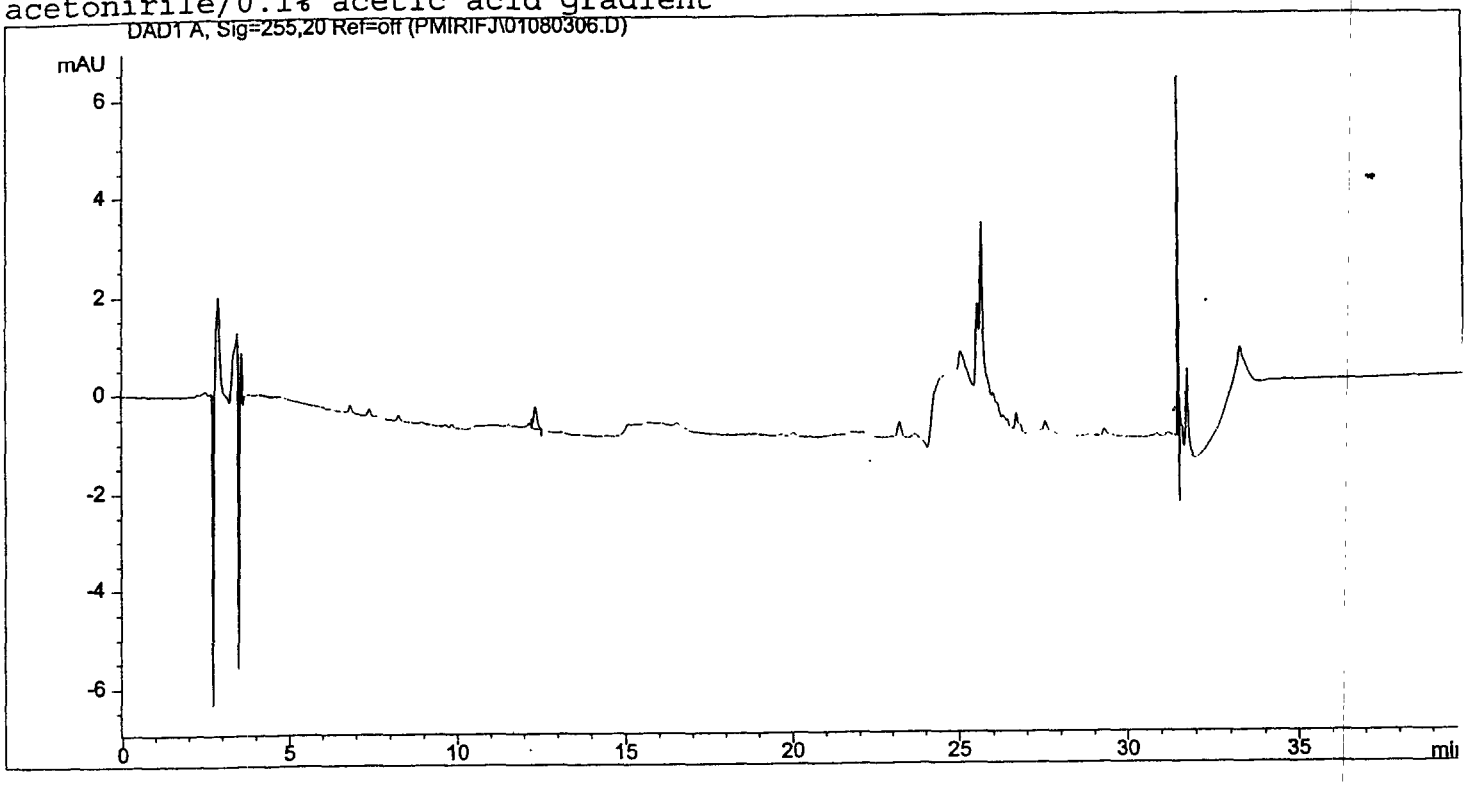
Injection Date : 1/8/03 5:59:40 PM
Sample Name : VII-179-10
Acq. Operator : mr

Seq. Line : 6
Vial : 6
Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 10:27:48 AM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



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External Standard Report

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Sorted By : Signal
Calib. Data Modified : 1/9/03 10:27:38 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.239		-	-	-		puerarin
9.808		-	-	-		daidzin
12.124		-	-	-		genistin
16.516		-	-	-		daidzein
20.046		-	-	-		genistein

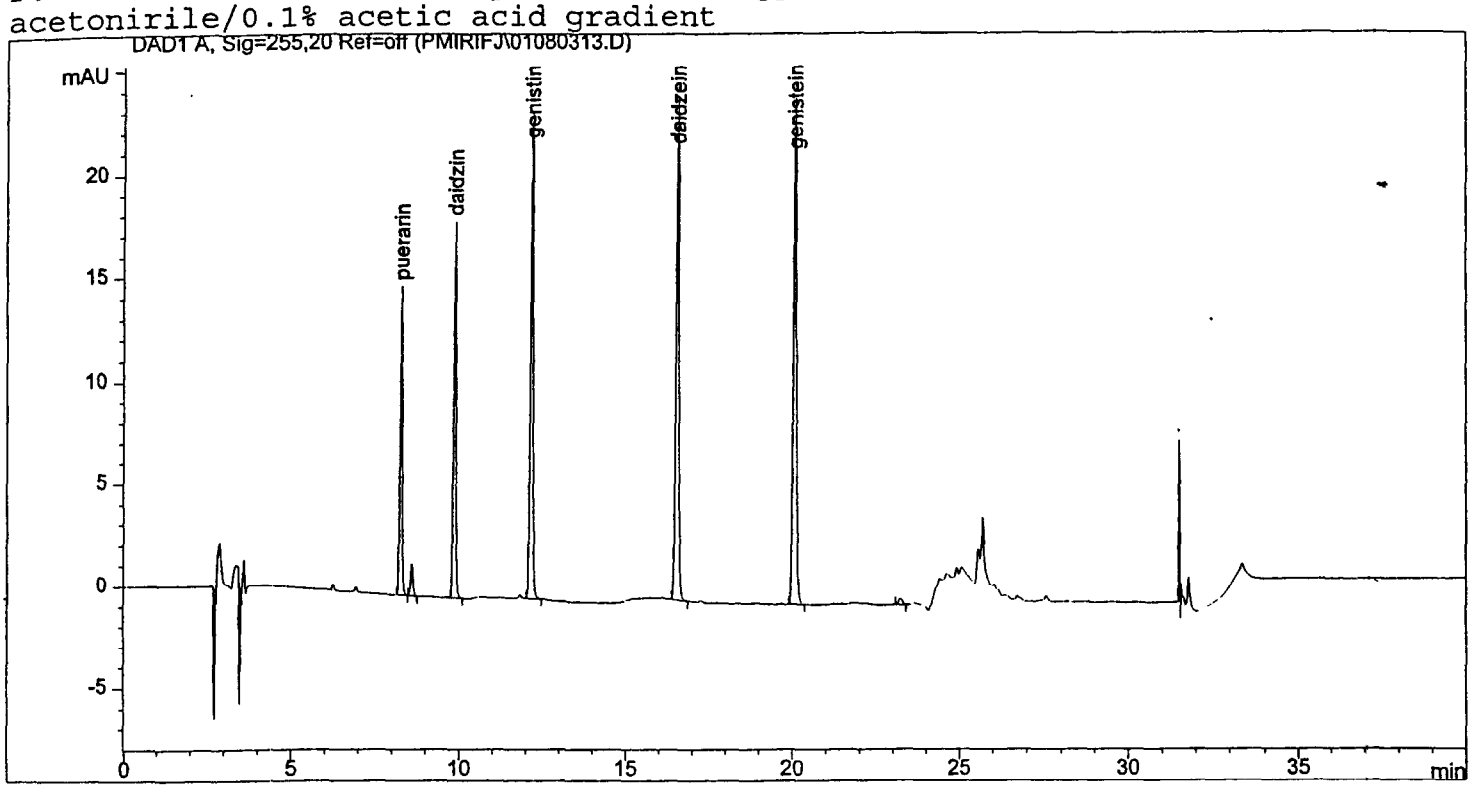
no page 2, end of report

cal check @ 5 ug/mL

Injection Date	: 1/8/03 10:47:32 PM	Seq. Line :	13
Sample Name	: stdIV-179-10	Vial :	3
Acq. Operator	: mr	Inj :	1
		Inj Volume :	5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/6/02 10:13:43 AM by mr
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 1/9/03 10:27:48 AM by mr
(modified after loading)

P. mirifica method development on Prodigy C18, 250 X 4.5mm, with acetonirile/0.1% acetic acid gradient



External Standard Report

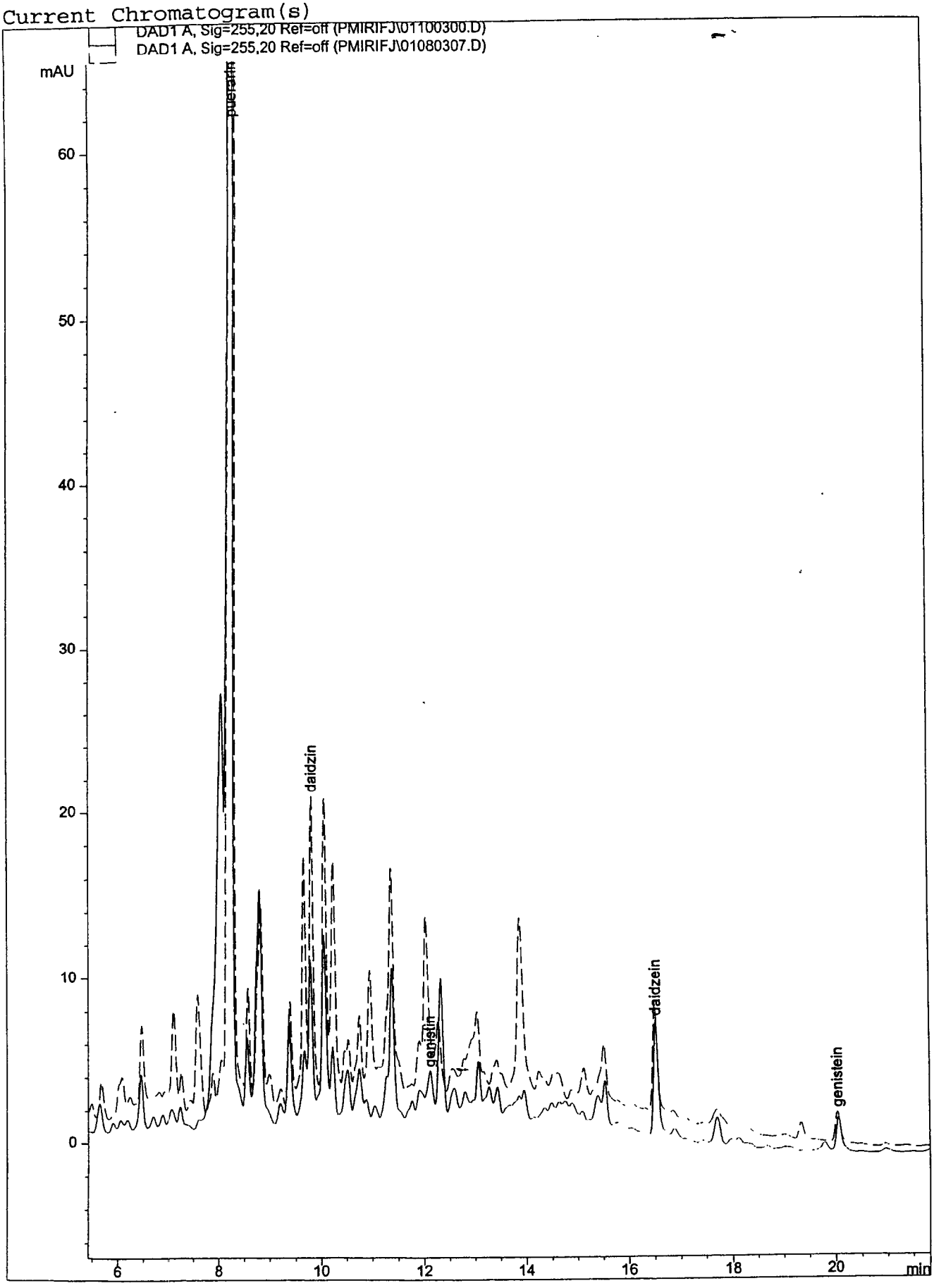
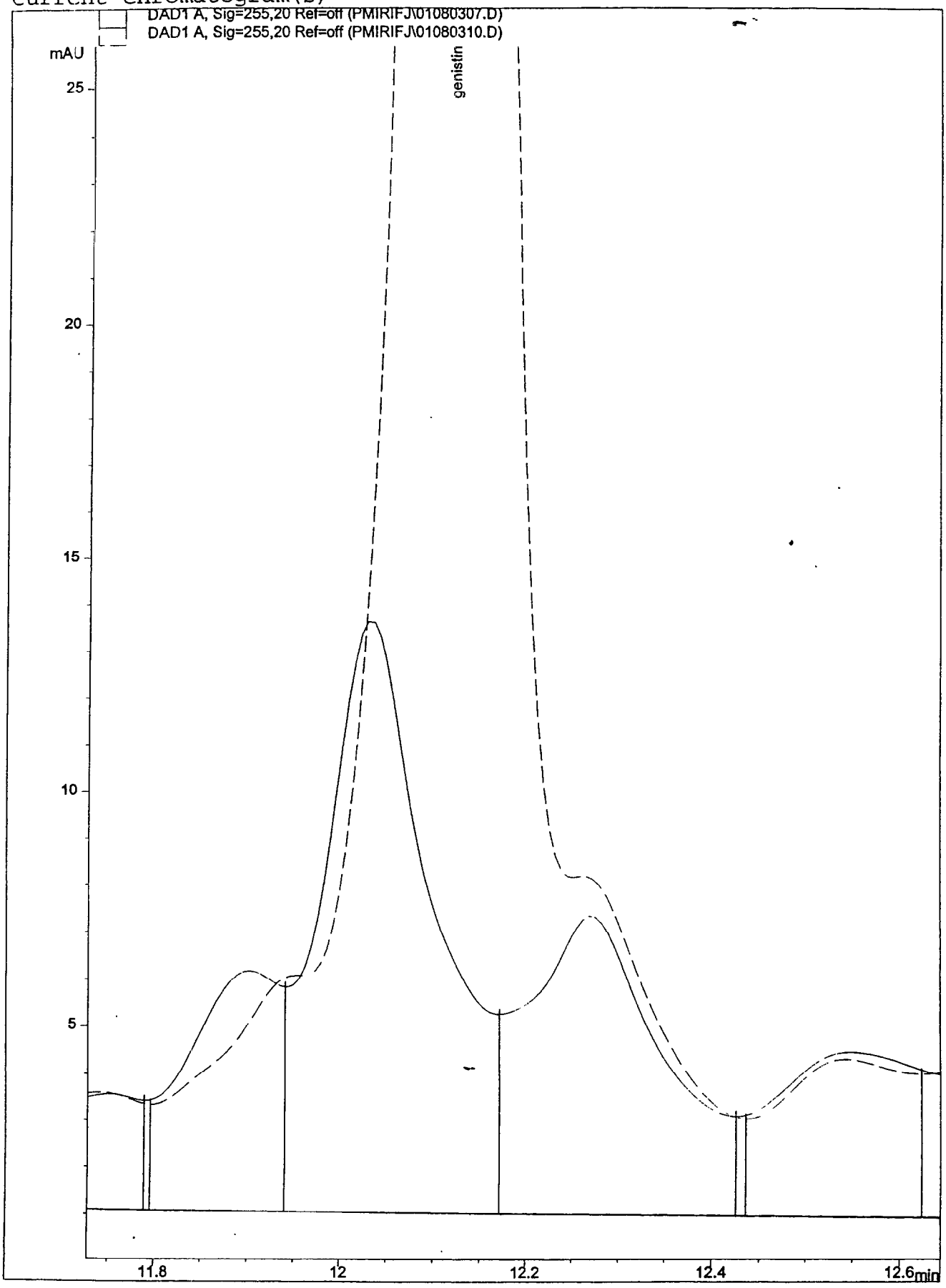
Sorted By : Signal
Calib. Data Modified : 1/9/03 10:27:38 AM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=255,20 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name	
8.228	BB	73.25765	5.85448e-2	4.28885		puerarin	100.7%
9.815	BB	93.65222	4.86416e-2	4.55540		daidzin	100.6%
12.124	BB	128.93810	4.39021e-2	5.66065		genistin	101.4%
16.507	BB	150.67288	3.65266e-2	5.50357		daidzein	101.0%
20.036	BB	162.49530	3.12931e-2	5.08499		genistein	101.7%

no page 2, end of report

Current Chromatogram(s)



Current Chromatogram(s)