PM FLUID EXTRACT ACCURACY
AND
PRECISION STUDY

VII-169-6		spike recoveries			
Analyte	**sample mean**	**sample wt.**	**amount**	**spike**	**total**
puerarin	942.42	0.8916	840.26	0.0	840.3
daidzin	479.11	0.8916	427.17	202.0	629.2
genistin	183.58	0.8916	163.68	0.0	163.7
daidzein	209.17	0.8916	186.50	218.0	404.5
genistein	155.91	0.8916	139.01	418.0	557.0
Analyte	**found**	**sample wt.**	**amount**	**recovery**	
puerarin	838.04	0.8916	747.20	88.9	
daidzin	653.27	0.8916	582.46	92.6	
genistin	163.51	0.8916	145.79	89.1	
daidzein	448.08	0.8916	399.51	98.8	
genistein	574.91	0.8916	512.59	92.0	

VII-169-7		spike recoveries			
Analyte	**sample mean**	**sample wt.**	**amount**	**spike**	**total**
puerarin	942.42	0.9746	918.48	0.0	918.5
daidzin	479.11	0.9746	466.94	202.0	668.9
genistin	183.58	0.9746	178.92	0.0	178.9
daidzein	209.17	0.9746	203.86	218.0	421.9
genistein	155.91	0.9746	151.95	418.0	569.9
Analyte	**found**	**sample wt.**	**amount**	**recovery**	
puerarin	846.8	0.9746	825.29	89.9	
daidzin	638.89	0.9746	622.66	93.1	
genistin	160.803	0.9746	156.72	87.6	
daidzein	428.156	0.9746	417.28	98.9	
genistein	535.667	0.9746	522.06	91.6	

VII-169-8		spike recoveries			
Analyte	**sample mean**	**sample wt.**	**amount**	**spike**	**total**
puerarin	942.42	0.9678	912.07	0.0	912.1
daidzin	479.11	0.9678	463.68	202.0	665.7
genistin	183.58	0.9678	177.67	0.0	177.7
daidzein	209.17	0.9678	202.43	218.0	420.4
genistein	155.91	0.9678	150.89	418.0	568.9
Analyte	**found**	**sample wt.**	**amount**	**recovery**	
puerarin	848.576	0.9678	821.25	90.0	
daidzin	643.789	0.9678	623.06	93.6	
genistin	168.345	0.9678	162.92	91.7	
daidzein	429.666	0.9678	415.83	98.9	
genistein	542.048	0.9678	524.59	92.2	

VII-169-9		spike recoveries			
Analyte	**sample mean**	**sample wt.**	**amount**	**spike**	**total**
puerarin	942.42	0.9154	862.69	0.0	862.7
daidzin	479.11	0.9154	438.58	202.0	640.6
genistin	183.58	0.9154	168.05	0.0	168.0
daidzein	209.17	0.9154	191.47	218.0	409.5
genistein	155.91	0.9154	142.72	418.0	560.7
Analyte	**found**	**sample wt.**	**amount**	**recovery**	
puerarin	862.052	0.9154	789.12	91.5	
daidzin	649.948	0.9154	594.96	92.9	
genistin	166.056	0.9154	152.01	90.5	
daidzein	440.327	0.9154	403.08	98.4	
genistein	563.1	0.9154	515.46	91.9	

VII-169-10		spike recoveries			
Analyte	**sample mean**	**sample wt.**	**amount**	**spike**	**total**
puerarin	942.42	0.9068	854.59	0.0	854.6
daidzin	479.11	0.9068	434.46	202.0	636.5
genistin	183.58	0.9068	166.47	0.0	166.5
daidzein	209.17	0.9068	189.68	218.0	407.7
genistein	155.91	0.9068	141.38	418.0	559.4
Analyte	**found**	**sample wt.**	**amount**	**recovery**	
puerarin	840.389	0.9068	762.06	89.2	
daidzin	650.824	0.9068	590.17	92.7	
genistin	165.673	0.9068	150.23	90.2	
daidzein	444.307	0.9068	402.90	98.8	
genistein	570.291	0.9068	517.14	92.4	

VII-169-11		spike recoveries			
Analyte	**sample mean**	**sample wt.**	**amount**	**spike**	**total**
puerarin	942.42	0.9634	907.93	0.0	907.9
daidzin	479.11	0.9634	461.57	202.0	663.6
genistin	183.58	0.9634	176.86	0.0	176.9
daidzein	209.17	0.9634	201.51	218.0	419.5
genistein	155.91	0.9634	150.20	418.0	568.2
Analyte	**found**	**sample wt.**	**amount**	**recovery**	
puerarin	854.644	0.9634	823.36	90.7	
daidzin	641.283	0.9634	617.81	93.1	
genistin	165.137	0.9634	159.09	90.0	
daidzein	430.489	0.9634	414.73	98.9	
genistein	541.9	0.9634	522.07	91.9	

smith p. mirifica fluid extracts Accuracy Precision

FR Sample ID	Compound	Instrument Concentration (ug/ml)	Weight (g)	conc. ug/g
VII-169-6	puerarin	7.47193	0.8916	838.036
	daidzin	5.82458	0.8916	653.273
	genistin	1.45787	0.8916	163.512
	daidzein	3.99513	0.8916	448.085
	genistein	5.12591	0.8916	574.911
VII-169-7	puerarin	8.25290	0.9746	846.799
	daidzin	6.22661	0.9746	638.889
	genistin	1.56719	0.9746	160.803
	daidzein	4.17281	0.9746	428.156
	genistein	5.22061	0.9746	535.667
VII-169-8	puerarin	8.21252	0.9678	848.576
	daidzin	6.23059	0.9678	643.789
	genistin	1.62924	0.9678	168.345
	daidzein	4.15831	0.9678	429.666
	genistein	5.24594	0.9678	542.048
VII-169-9	puerarin	7.89122	0.9154	862.052
	daidzin	5.94962	0.9154	649.948
	genistin	1.52008	0.9154	166.056
	daidzein	4.03075	0.9154	440.327
	genistein	5.15462	0.9154	563.100
VII-169-10	puerarin	7.62065	0.9068	840.389
	daidzin	5.90167	0.9068	650.824
	genistin	1.50232	0.9068	165.673
	daidzein	4.02898	0.9068	444.307
	genistein	5.17140	0.9068	570.291
VII-169-11	puerarin	8.23364	0.9634	854.644
	daidzin	6.17812	0.9634	641.283
	genistin	1.59093	0.9634	165.137
	daidzein	4.14733	0.9634	430.489
	genistein	5.22066	0.9634	541.900

VOLUME IN ML
100

dilution factor
1

		average	std. dev.	RSD
precision	puerarin	848.416	8.939	1.054
	daidzin	646.334	5.810	0.899
	genistin	164.921	2.552	1.547
	daidzein	436.838	8.504	1.947
	genistein	554.653	16.783	3.026

		average	std. dev.		RSD
accuracy	puerarin	90.025	0.948		1.054
	daidzin	92.994	0.359		0.386
	genistin	89.836	1.390		1.548
	daidzein	98.785	0.179		0.181
	genistein	92.016	0.292		0.318

10

13/02 STD IV-10-1 P. mirifica Spike Mix ?? genistein 12-

STD IV-9-13 Genistein ca. 1.08 mg/mL

Amount: 2.0 mL

STD III-195-17 Genistein ca. 1.01 mg/mL

Amount: 2.0 mL

STD IV-9-20 Daidzin ca. 1.01 mg/mL

Amount: 2.0 mL 12-

STD III-196-1 Daidzein ca. 1.09 mg/mL

Amount: 2.0 mL

Solvent: SOL II-194-7 (MeOH/H_2O, 8:2)

F.V.: 10.0 mL

F.C.: ca. 0.2 mg/mL

Exp.: 4/31/03 unless demonstrated otherwise MR 12-

3-02 STD IV-10-15

FAME Internal Standard STOCK

Heptadecanoic Acid

STK# 20-165-012

QUAN: 100.5 mg

Nonanoic Acid

STK# 20-102-005

QUAN: 108.4 mg

SOLV: Hexane

STK# 21-107-009

FV: 50 mL

EXP: 5-3-03 AM

[redacted]

12/3/02 P. mirifica Fluid Extract
Accuracy & precission Study
spikes on 220109005

	Replicate	weight	spike STD III-10-1
VII-169-6	1	0.8916 g	1.0 mL
VII-169-7	2	0.9746 g	1.0 mL
VII-169-8	3	0.9678 g	1.0 mL
VII-169-9	4	0.9154 g	1.0 mL
VII-169-10	5	0.9068 g	1.0 mL
VII-169-11	6	0.9634 g	1.0 mL
VII-169-12	Method Blank	—	

weighed into 100 mL volumetrics
diluted to volume w/ SOL II-196-13 (EtOH/H_2O, 8:2)

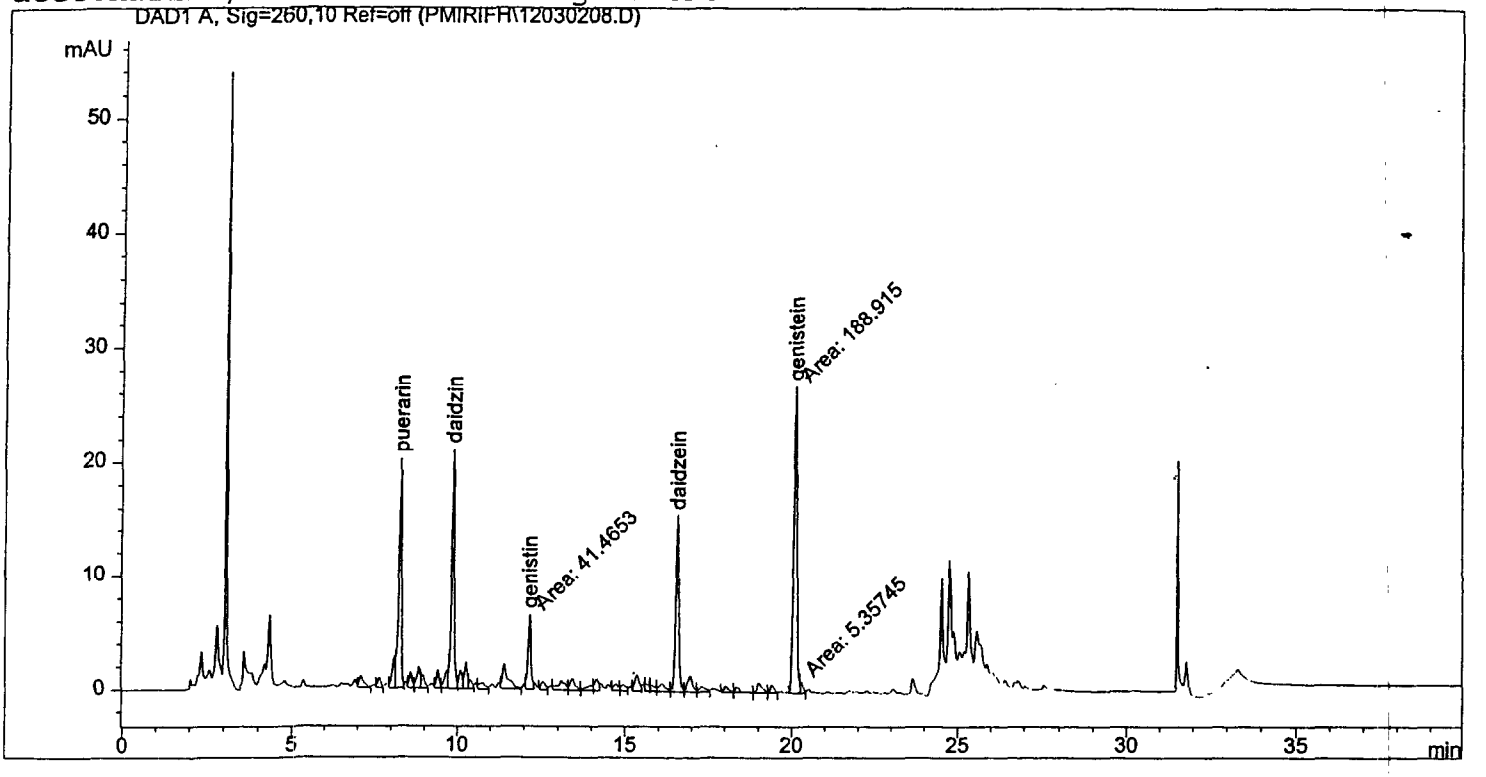
==========

Injection Date : 12/3/02 9:13:58 PM
Sample Name : VII-169-8
Acq. Operator : mr

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

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 11/22/02 4:30:12 PM by AM
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/4/02 1:01:48 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 : 12/4/02 12:59:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.240	VV	122.43800	6.70749e-2	8.21252		puerarin
9.817	VV	123.44065	5.04744e-2	6.23059		daidzin
12.145	FM	41.46531	3.92915e-2	1.62924		genistin
16.533	VV	104.51815	3.97856e-2	4.15831		daidzein
20.065	MF	188.91533	2.77687e-2	5.24594		genistein

Totals : 25.47660

no page 2, end of report

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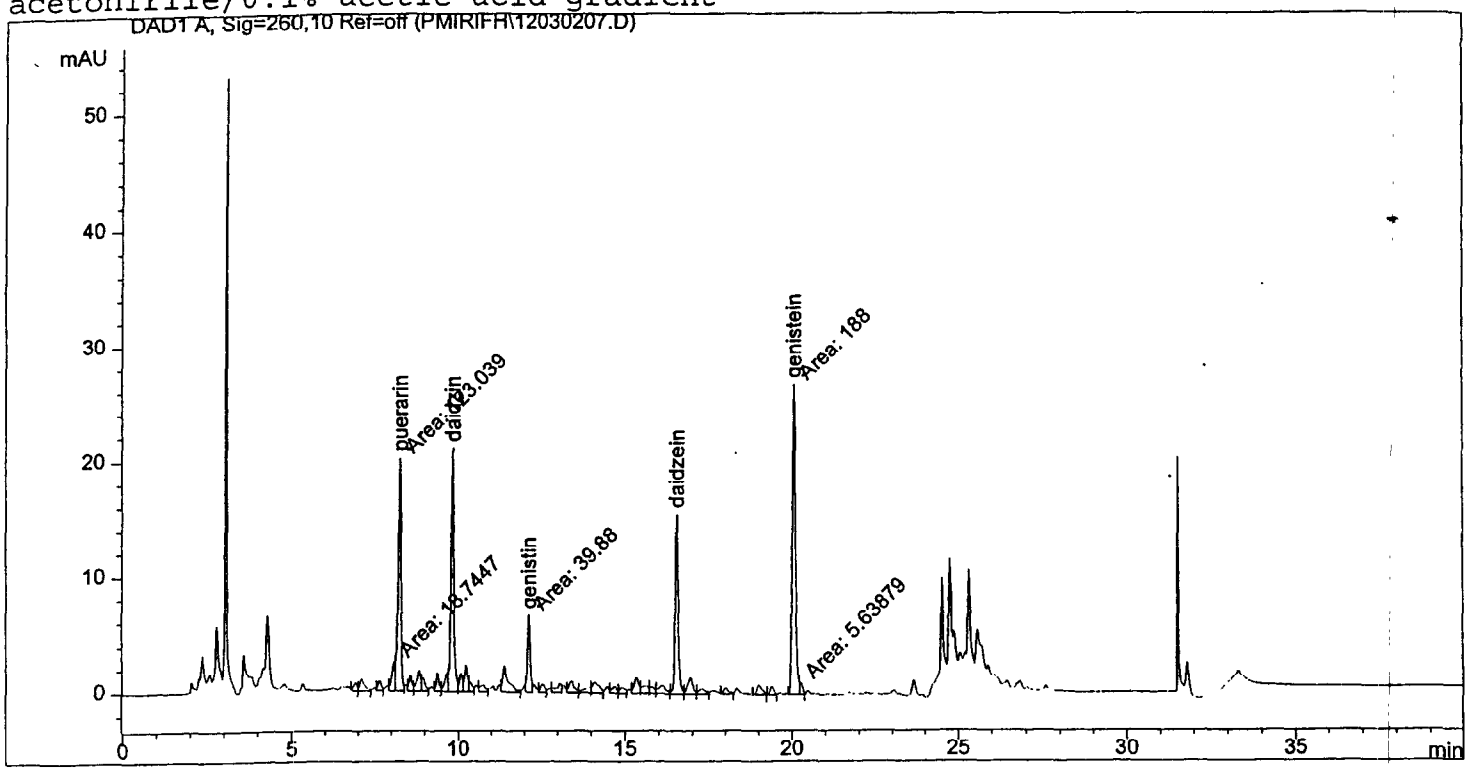
Injection Date : 12/3/02 8:32:48 PM
Sample Name : VII169-7
Acq. Operator : mr

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

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 11/22/02 4:30:12 PM by AM
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/4/02 1:01:48 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 : 12/4/02 12:59:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.217	FM	123.03886	6.70756e-2	8.25290		puerarin
9.795	VV	123.36154	5.04744e-2	6.22661		daidzin
12.116	FM	39.88000	3.92976e-2	1.56719		genistin
16.507	VV	104.88260	3.97856e-2	4.17281		daidzein
20.042	MF	188.00041	2.77691e-2	5.22061		genistein

Totals : 25.44011

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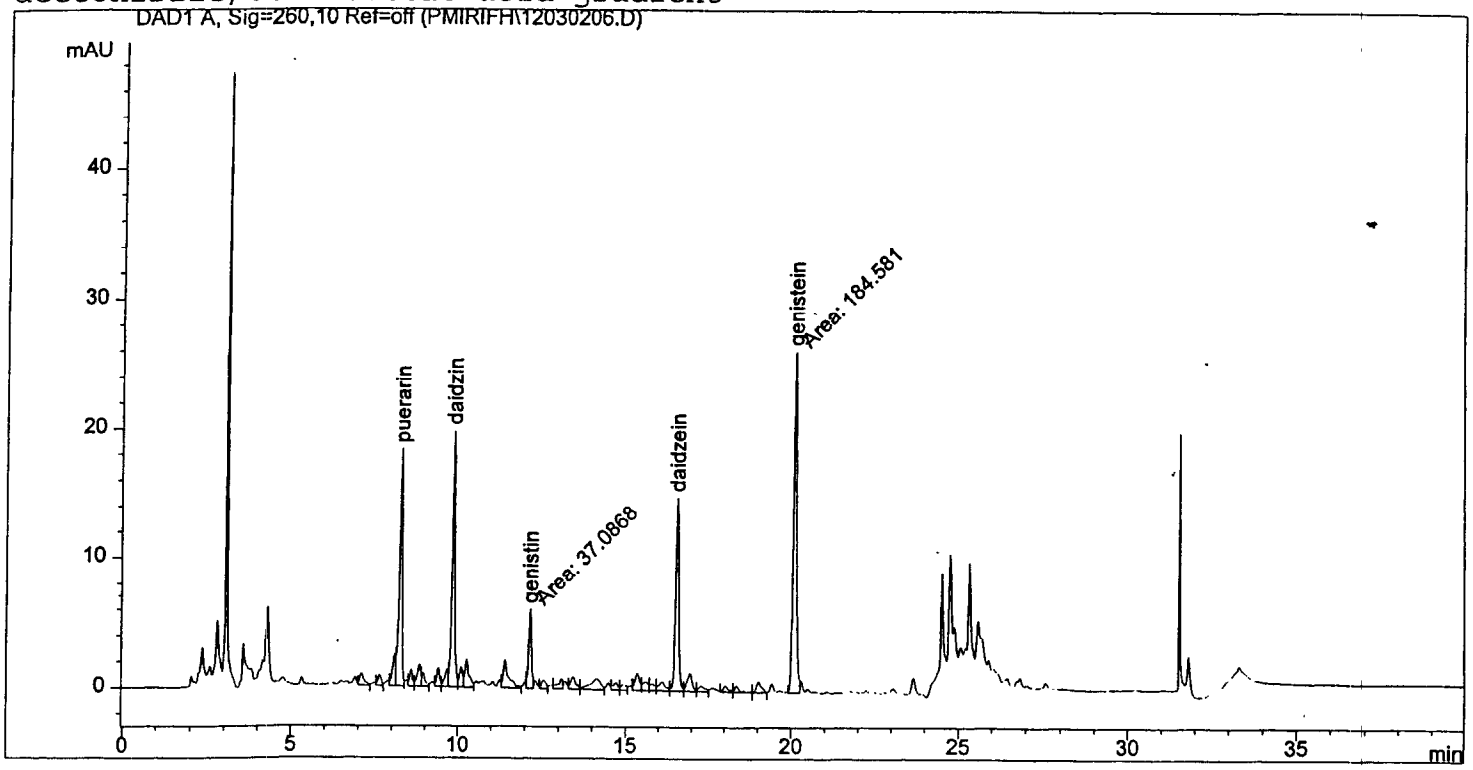
Injection Date : 12/3/02 7:51:41 PM
Sample Name : VII-169-6
Acq. Operator : mr

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

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 11/22/02 4:30:12 PM by AM
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/4/02 1:01:48 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 : 12/4/02 12:59:35 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.222	VV	111.41800	6.70622e-2	7.47193		puerarin
9.808	VV	115.38007	5.04816e-2	5.82458		daidzin
12.133	MF	37.08681	3.93095e-2	1.45787		genistin
16.529	VV	100.41650	3.97856e-2	3.99513		daidzein
20.065	MF	184.58066	2.77706e-2	5.12591		genistein

Totals : 23.87541

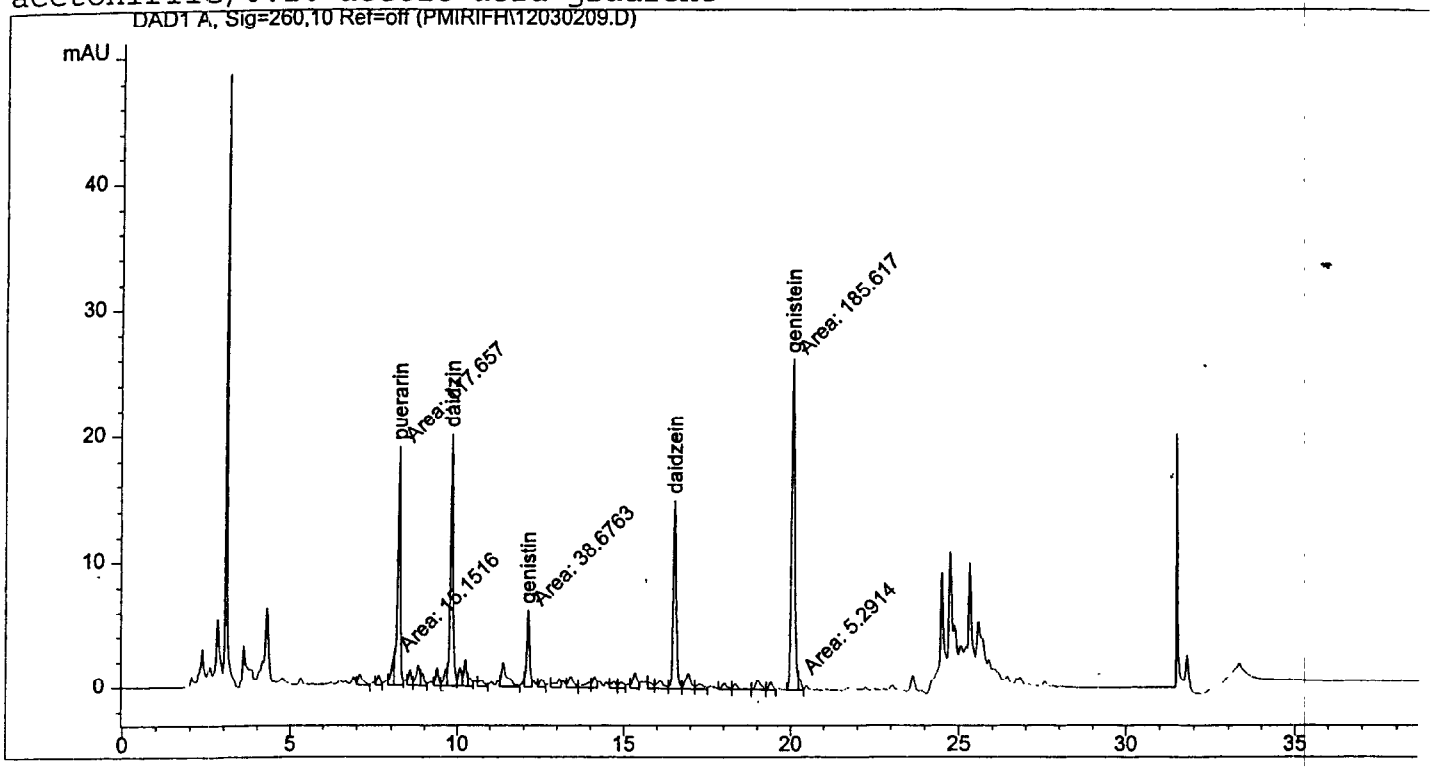
no page 2 end of report

Injection Date : 12/3/02 9:55:10 PM
Sample Name : VII-169-9
Acq. Operator : mr

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

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 11/22/02 4:30:12 PM by AM
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/4/02 1:11:04 PM 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 : Wednesday, December 04, 2002 1:11:03 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.207	FM	117.65695	6.70697e-2	7.89122		puerarin
9.790	VV	117.86263	5.04793e-2	5.94962		daidzin
12.113	FM	38.67628	3.93025e-2	1.52008		genistin
16.510	VV	101.31201	3.97856e-2	4.03075		daidzein
20.047	MF	185.61736	2.77701e-2	5.15462		genistein

Totals : 24.54629

no page 2, end of report

a File C:\HPCHEM\1\DATA\PMIRIFH\12030210.D Sample Name: VII-169-1

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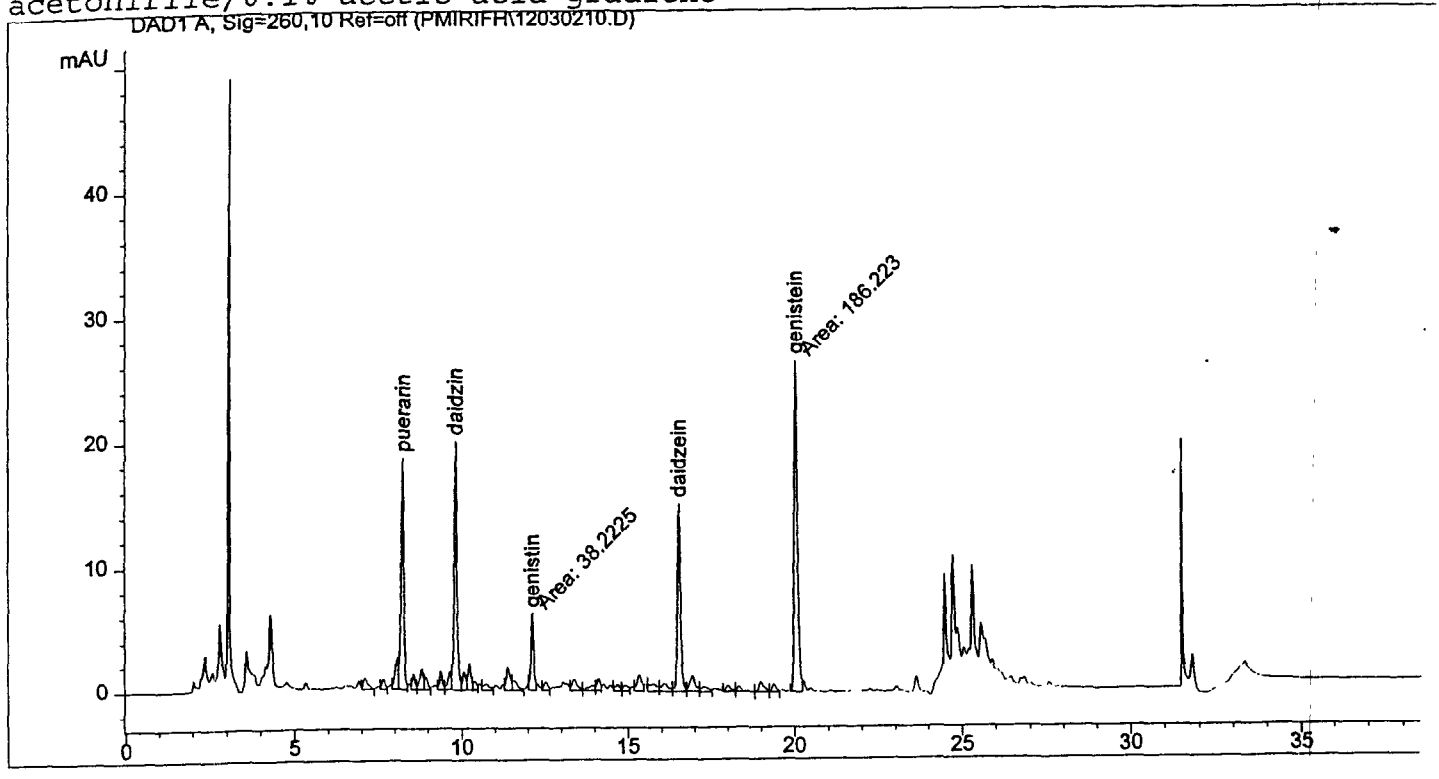
Injection Date : 12/3/02 10:36:19 PM
Sample Name : VII-169-10
Acq. Operator : mr

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

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 11/22/02 4:30:12 PM by AM
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/4/02 1:11:04 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 : Wednesday, December 04, 2002 1:11:03 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.216	VV	113.63090	6.70649e-2	7.62065		puerarin
9.796	VV	116.91059	5.04802e-2	5.90167		daidzin
12.113	FM	38.22255	3.93045e-2	1.50232		genistin
16.506	VV	101.26737	3.97856e-2	4.02898		daidzein
20.041	MF	186.22331	2.77699e-2	5.17140		genistein

Totals : 24.22501

no page 2, end of report

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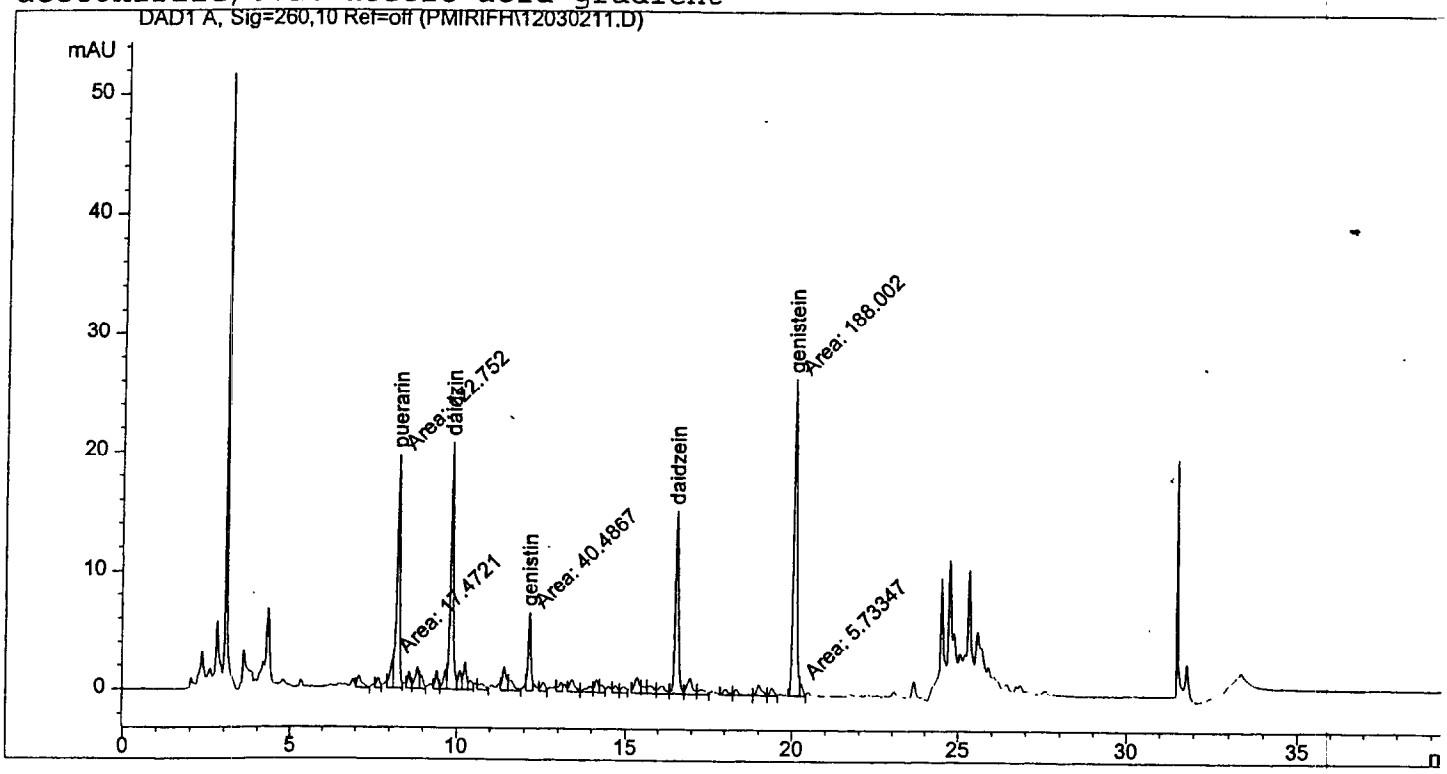
Injection Date : 12/3/02 11:17:29 PM
Sample Name : VII-169-11
Acq. Operator : mr

Seq. Line : 11
Vial : 11
Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 11/22/02 4:30:12 PM by AM
Analysis Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 12/4/02 1:11:04 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 : Wednesday, December 04, 2002 1:11:03 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name
8.228	FM	122.75225	6.70753e-2	8.23364		puerarin
9.809	VV	122.39891	5.04753e-2	6.17812		daidzin
12.137	FM	40.48669	3.92952e-2	1.59093		genistin
16.521	VV	104.24216	3.97856e-2	4.14733		daidzein
20.055	MF	188.00246	2.77691e-2	5.22066		genistein

Totals : 25.37068

Calibration Table

Calib. Data Modified : 12/19/02 1:27:45 PM

Calculate : External Standard
Based on : Peak Area

Rel. Reference Window : 1.000 %
Abs. Reference Window : 0.000 min
Rel. Non-ref. Window : 1.000 %
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 : Included
Weight : Equal

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)

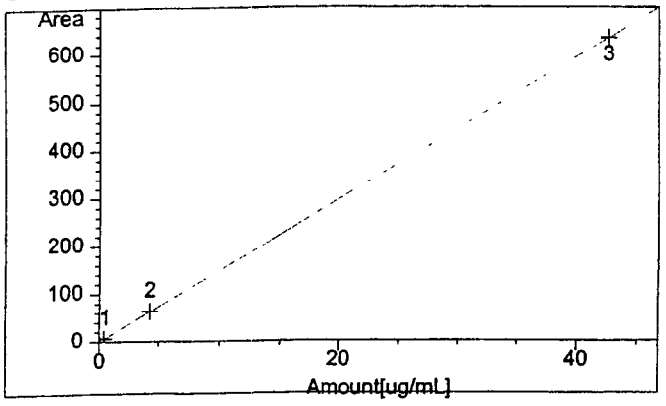
Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Sig	Lvl	Amount [ug/mL]	Area	Amt/Area	Ref	Grp	Name
8.233	1	1	4.26000e-1	6.70093	6.35733e-2			puerarin
		2	4.26000	63.48389	6.71036e-2			
		3	42.60000	634.14099	6.71775e-2			
9.805	1	1	4.53000e-1	8.97423	5.04778e-2			daidzin
		2	4.53000	89.41963	5.06600e-2			
		3	45.30000	899.10693	5.03833e-2			
12.126	1	1	5.58000e-1	14.25866	3.91341e-2			genistin
		2	5.58000	142.22726	3.92330e-2			
		3	55.80000	1425.53918	3.91431e-2			
16.516	1	1	5.45000e-1	13.44036	4.05495e-2			daidzein
		2	5.45000	137.26840	3.97032e-2			
		3	54.50000	1369.81592	3.97864e-2			
20.049	1	1	5.05000e-1	17.49744	2.88614e-2			genistein
		2	5.05000	182.06348	2.77376e-2			
		3	50.50000	1822.08203	2.77155e-2			

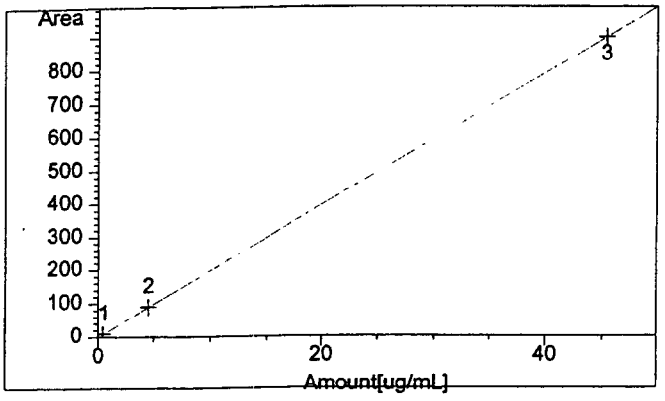
Peak Sum Table

No Entries in table

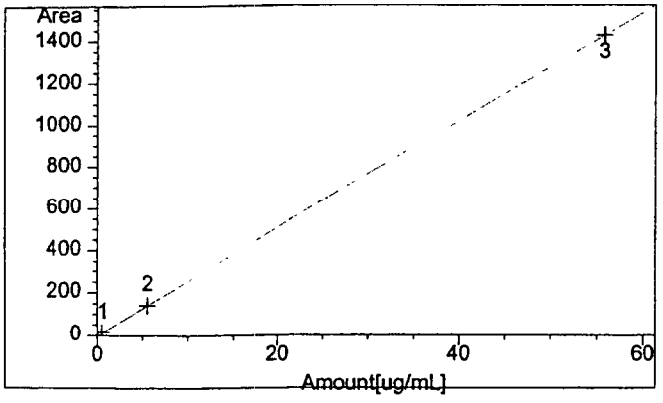
Calibration Curves



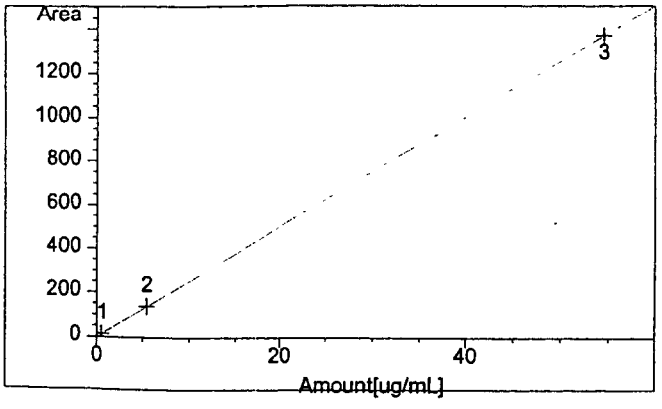
puerarin at exp. RT: 8.233
DAD1 A, Sig=260,10 Ref=off
Correlation: 1.00000
Residual Std. Dev.: 0.18876
Formula: y = mx + b
m: 14.88231
b: 1.50234e-1
x: Amount [ug/mL]
y: Area



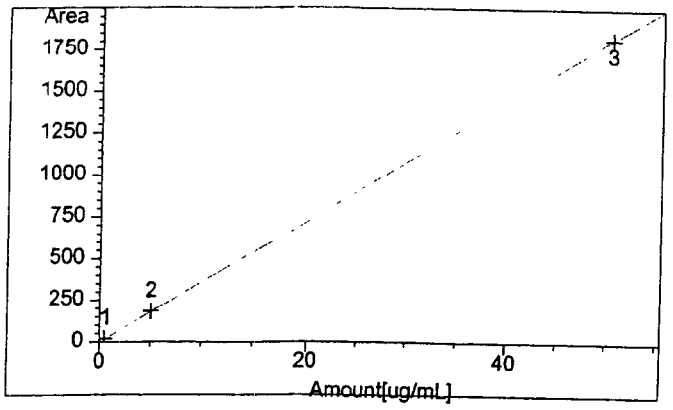
daidzin at exp. RT: 9.805
DAD1 A, Sig=260,10 Ref=off
Correlation: 1.00000
Residual Std. Dev.: 0.28720
Formula: y = mx + b
m: 19.85072
b: -1.63206e-1
x: Amount [ug/mL]
y: Area



genistin at exp. RT: 12.126
DAD1 A, Sig=260,10 Ref=off
Correlation: 1.00000
Residual Std. Dev.: 0.19484
Formula: y = mx + b
m: 25.54875
b: -1.03417e-1
x: Amount [ug/mL]
y: Area



daidzein at exp. RT: 16.516
DAD1 A, Sig=260,10 Ref=off
Correlation: 1.00000
Residual Std. Dev.: 0.27207
Formula: y = mx + b
m: 25.13471
b: 1.17449e-4
x: Amount [ug/mL]
y: Area



genistein at exp. RT: 20.049
DAD1 A, Sig=260,10 Ref=off
Correlation: 1.00000
Residual Std. Dev.: 0.37907
Formula: y = mx + b
m: 36.08701
b: -3.03664e-1
x: Amount [ug/mL]
y: Area

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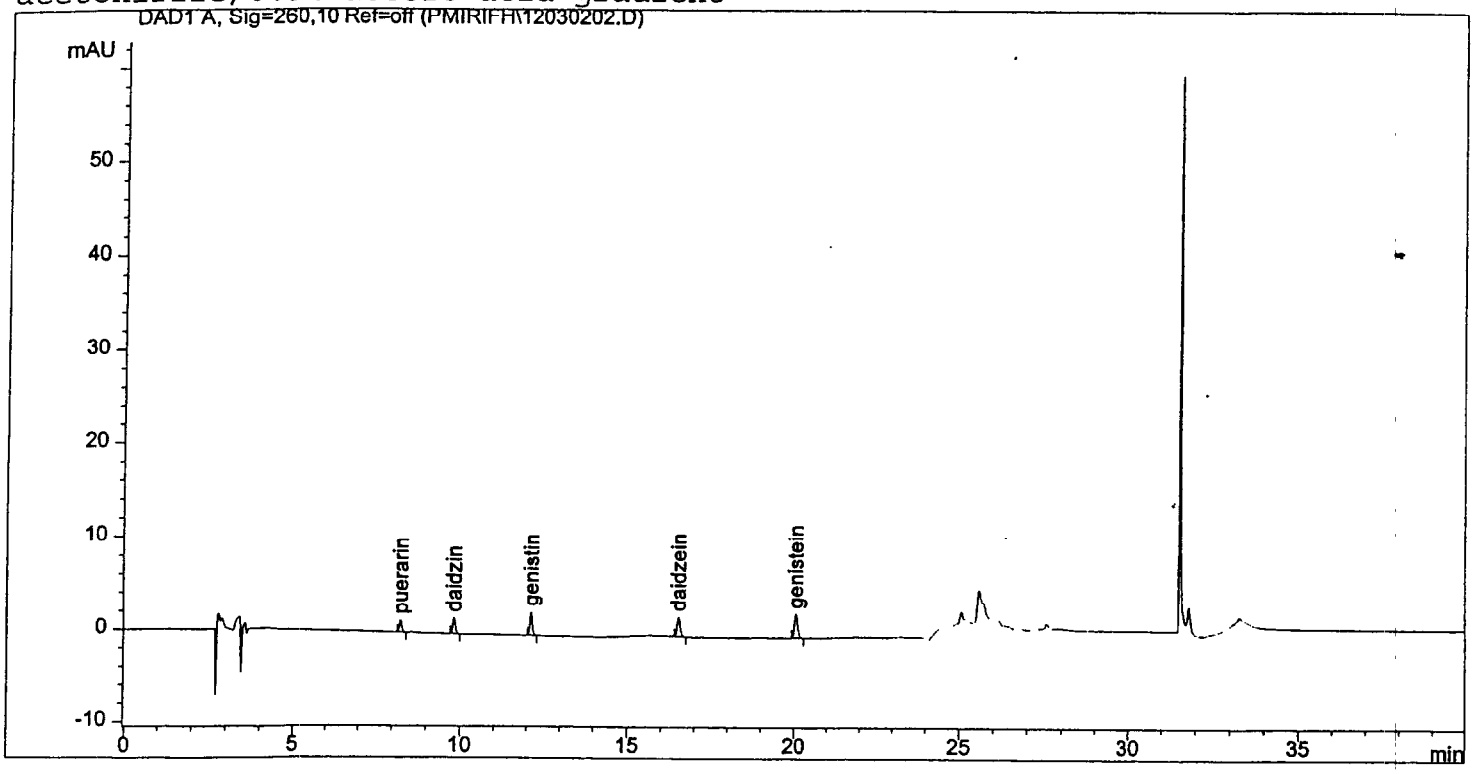
Injection Date : 12/3/02 5:07:12 PM
Sample Name : 0.5 std
Acq. Operator : mr

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

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 11/22/02 4:30:12 PM by AM
Analysis Method : C:\HPCHEM\1\METHODS\MIRIF3.M
Last changed : 12/19/02 1:27:54 PM by mr
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 : 12/19/02 1:27:45 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name	error
8.226	BB	6.70093	6.56874e-2	4.40166e-1		puerarin	3.3%
9.802	PB	8.97423	5.12922e-2	4.60308e-1		daidzin	1.6%
12.116	BB	14.25866	3.94247e-2	5.62144e-1		genistin	0.7%
16.506	BB	13.44036	3.97853e-2	5.34728e-1		daidzein	-1.9%
20.043	BB	17.49744	2.81917e-2	4.93283e-1		genistein	-2.3%
Totals :				2.49063			

Results obtained with enhanced integrator!

no page 2. end of report.

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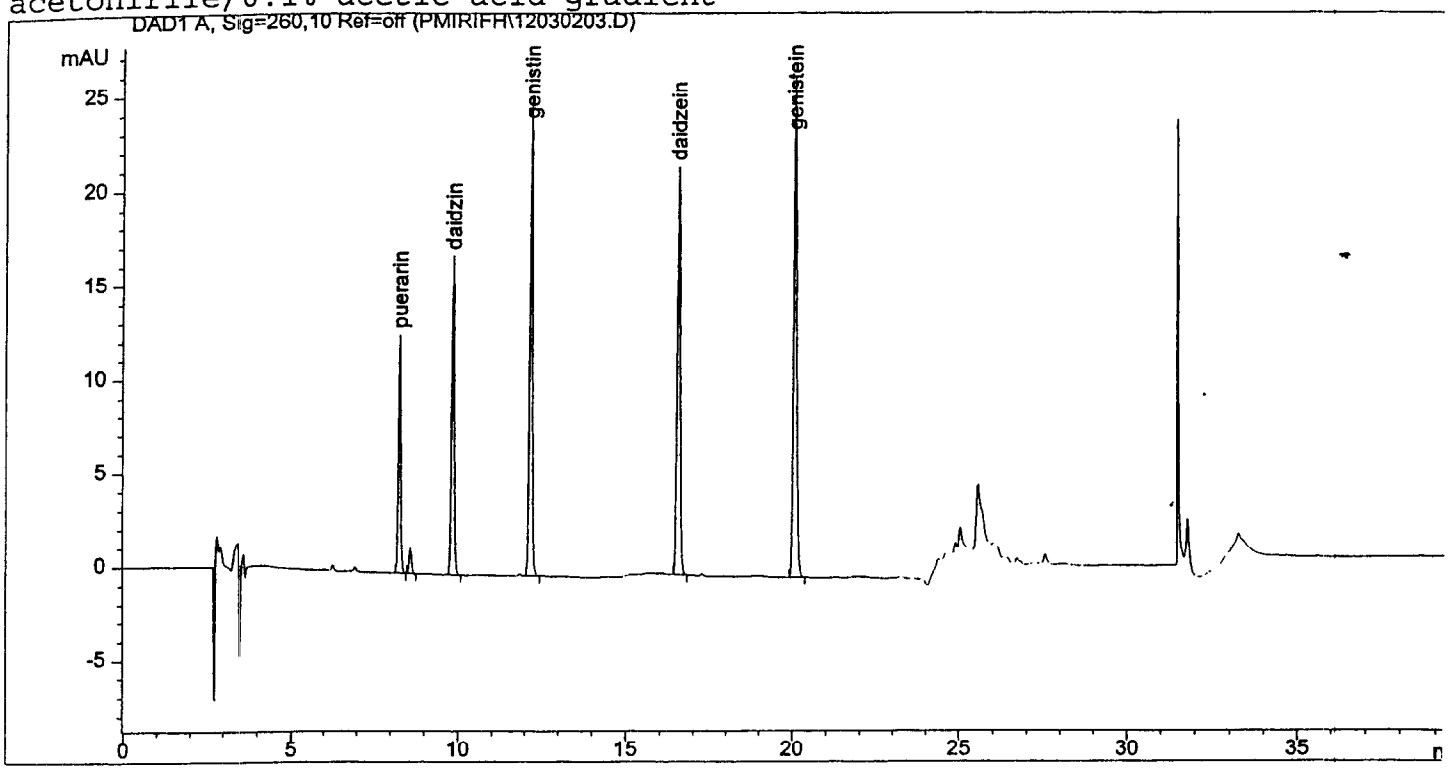
Injection Date : 12/3/02 5:48:18 PM
Sample Name : 5.0 std
Acq. Operator : mr

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

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 11/22/02 4:30:12 PM by AM
Analysis Method : C:\HPCHEM\1\METHODS\MIRIF3.M
Last changed : 12/19/02 1:27:54 PM by mr
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 : 12/19/02 1:27:45 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name	error
8.241	BB	63.48389	6.70349e-2	4.25563		puerarin	-0.1 %
9.818	BB	89.41963	5.04680e-2	4.51283		daidzin	-0.4 %
12.145	BB	142.22726	3.91693e-2	5.57094		genistin	-0.2 %
16.541	BB	137.26840	3.97856e-2	5.46130		daidzein	0.2 %
20.076	BB	182.06348	2.77570e-2	5.05354		genistein	0.1 %
Totals :				24.85425			

Results obtained with enhanced integrator!

no page 2, end of report

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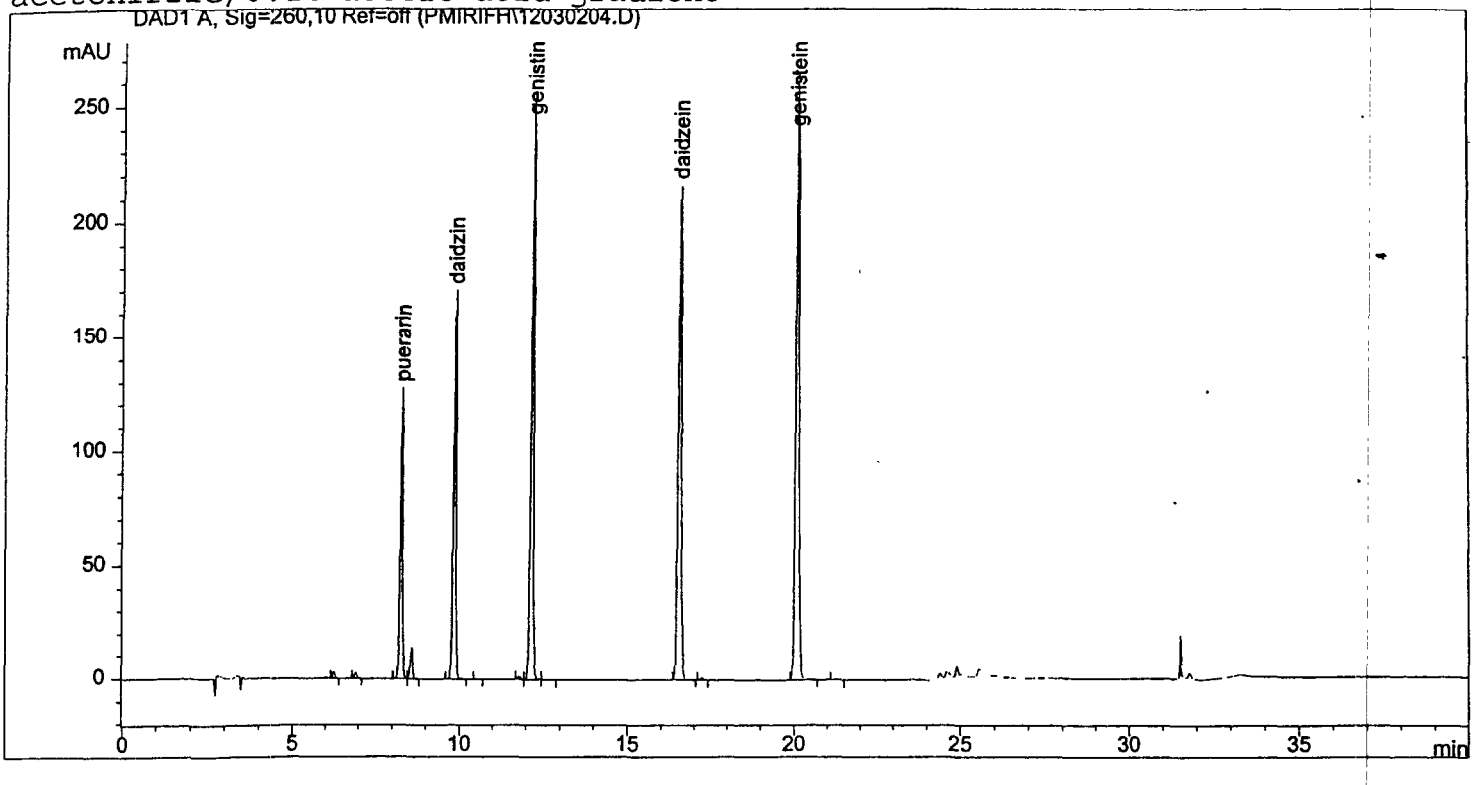
Injection Date : 12/3/02 6:29:23 PM Seq. Line : 4
Sample Name : 50.0 std Vial : 4
Acq. Operator : mr Inj : 1
Inj Volume : 5 µl

Acq. Method : C:\HPCHEM\1\METHODS\MIRIFIC3.M
Last changed : 11/22/02 4:30:12 PM by AM
Analysis Method : C:\HPCHEM\1\METHODS\MIRIF3.M
Last changed : 12/19/02 1:27:54 PM by mr
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 : 12/19/02 1:27:45 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: DAD1 A, Sig=260,10 Ref=off

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ug/mL]	Grp	Name	error
8.233	VV	634.14099	6.71780e-2	42.60029		puerarin	< .01 %
9.805	PB	899.10693	5.03852e-2	45.30164		daidzin	< .01 %
12.126	VV	1425.53918	3.91437e-2	55.80086		genistin	< .01 %
16.516	BB	1369.81592	3.97856e-2	54.49897		daidzein	< .01 %
20.049	BB	1822.08203	2.77154e-2	50.49976		genistein	< .01 %

Totals : 248.70154

Results obtained with enhanced integrator!

no page 2; end of report