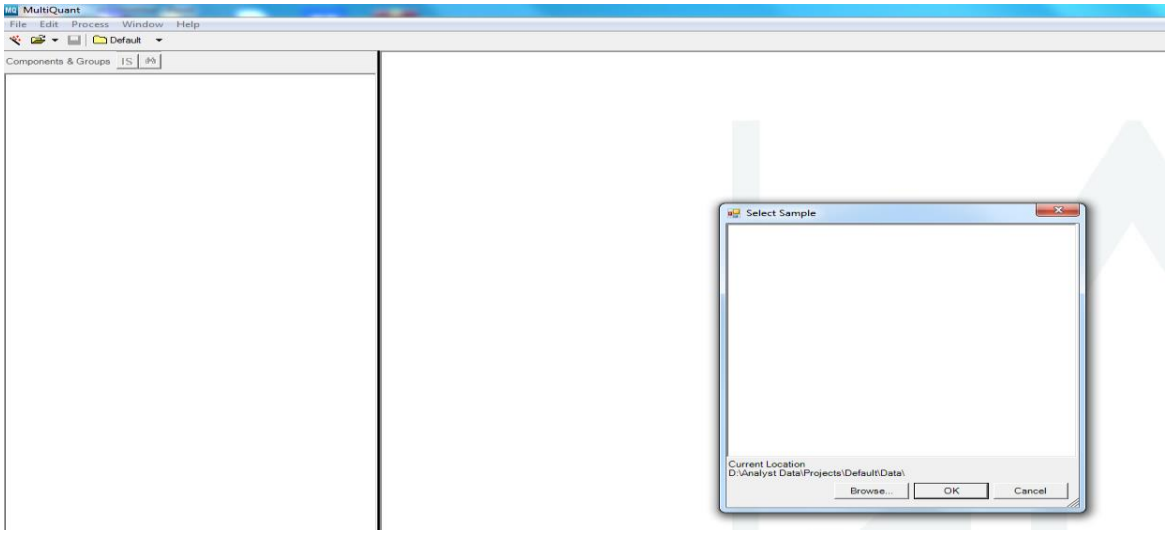
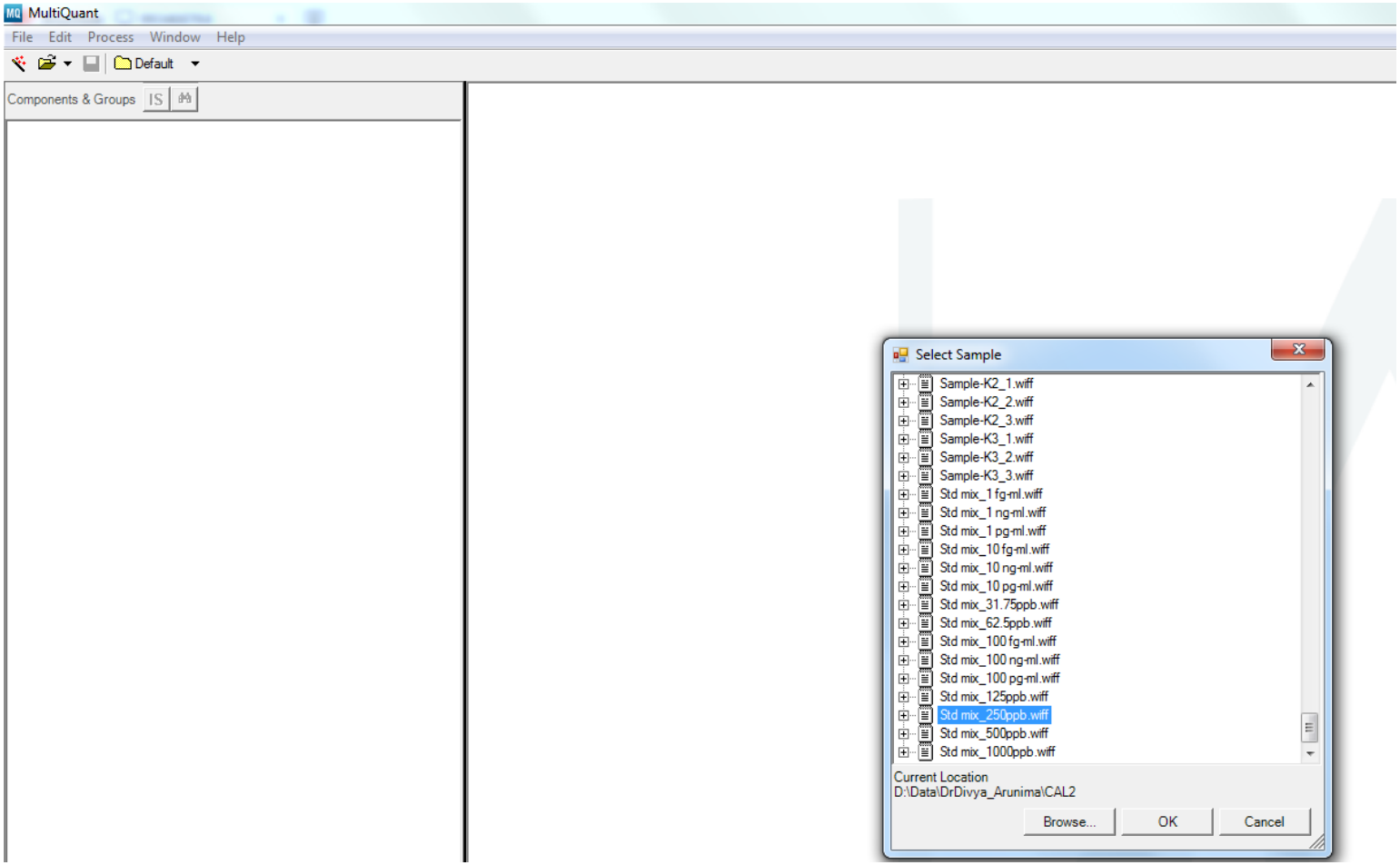
Multiquant for quantification of small molecules

Prepared by Amrutha N., Pallavi H.U., Dr. Ankit J., Dr. Nirpendra S.

1. Open **Multiquant**
2. You need to create two things; **New quantitation method** and **New results table**
   Go to File > New quantitation method

3. **Browse** your data files and pick any file in the medium concentration range.





- Before selecting this file, make sure to check the chromatogram, peak shape RT etc. as your method will be based on this particular file.

MultiQuant - [[MQ4] Untitled Method]

File Edit Process Window Help

Default

Components & Groups | IS

Components | Integration & Regression | Outlier Settings

Experiment: 1 MRM (14 transitions)

Row	IS	Name	Group	IS Name	Q1 / Q3
1		Naringenin			273.0 / 153.1
2		Naringenin			273.0 / 147.1
3		Liquiritigenin			257.0 / 137.0
4		Liquiritigenin			257.0 / 147.0
5		Daidzein			255.0 / 199.1
6		Daidzein			255.0 / 181.0
7		Formononetin			269.0 / 237.0
8		Formononetin			269.0 / 197.0
9		AGPA-13			299.0 / 267.0
10		AGPA-13			299.0 / 148.0
11		AGPA-14			285.0 / 225.1
12		AGPA-14			285.0 / 213.1
13		Biochanin A			285.0 / 225.1 (2)
14		Biochanin A			285.0 / 213.0
15					

- A list of all the standards and their transitions from .wiff file will be displayed
- Replicate names corresponds to the number of transitions used for one single molecule.
- But in order to process further, you need to edit the name like Naringenin, Naringenin _1 etc.

Components | Integration & Regression | Outlier Settings

Experiment: 1 MRM (14 transitions)

Row	IS	Name
1		Naringenin
2		Naringenin_1
3		Liquiritigenin
4		Liquiritigenin_1
5		Daidzein
6		Daidzein_1
7		Formononetin
8		Formononetin_1
9		AGPA-13
10		AGPA-13_1
11		AGPA-14
12		AGPA-14_1
13		Biochanin A
14		Biochanin A_1
15		

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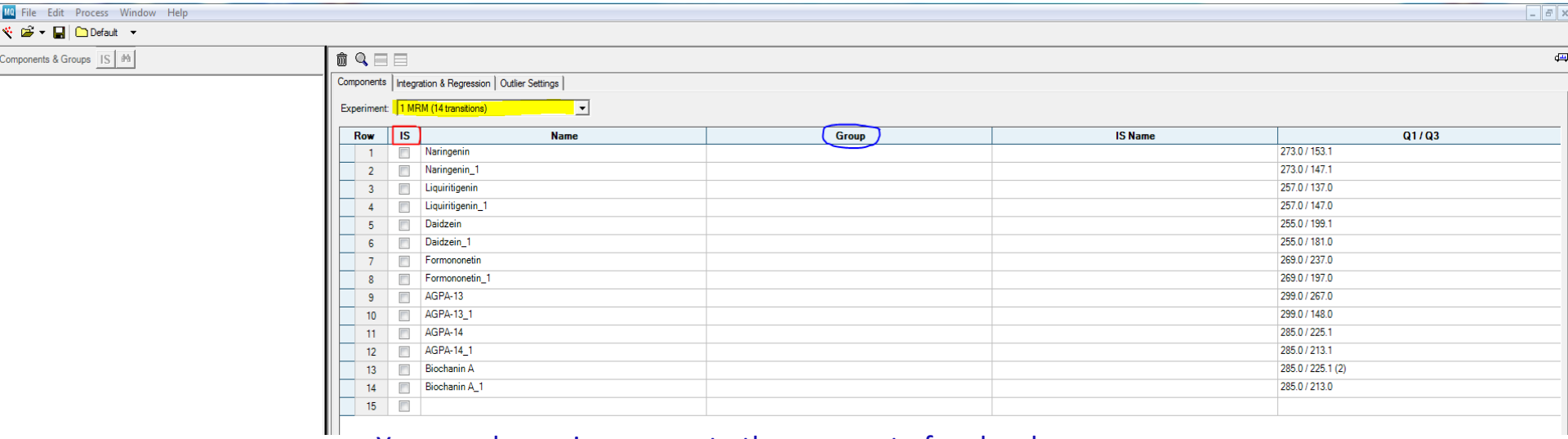
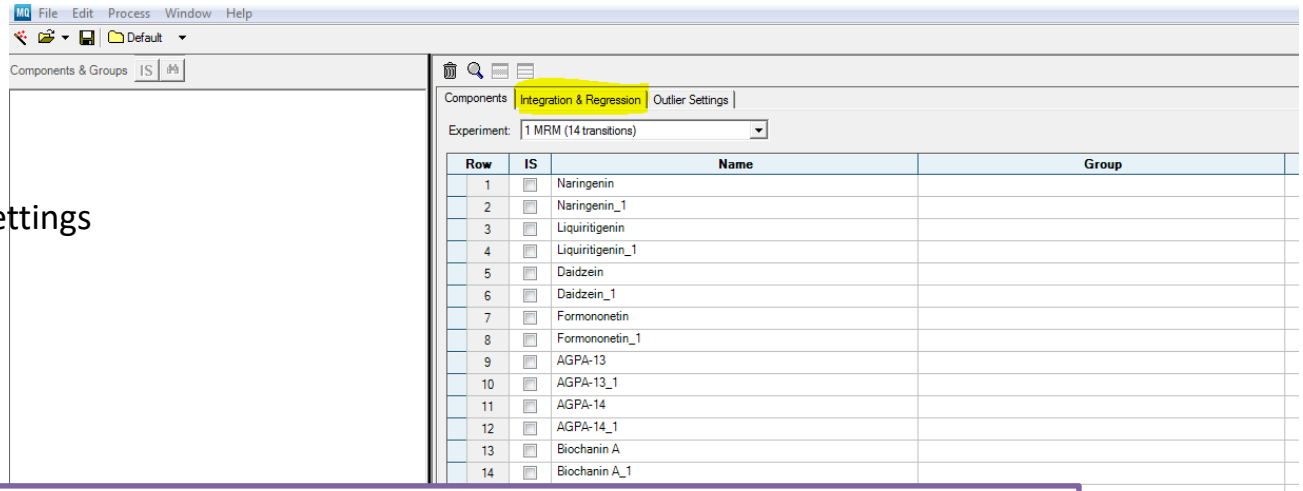
In the Experiment Tab, depending upon your experiment and information in the .wiff file, it will display transitions in the positive mode and in the negative mode. Click the drop down box



Row	IS	Name	Group	IS Name	Q1 / Q3
1		Naringenin			273.0 / 153.1
2		Naringenin_1			273.0 / 147.1
3		Liquiritigenin			257.0 / 137.0
4		Liquiritigenin_1			257.0 / 147.0
5		Daidzein			255.0 / 199.1
6		Daidzein_1			255.0 / 181.0
7		Formononetin			269.0 / 237.0
8		Formononetin_1			269.0 / 197.0
9		AGPA-13			299.0 / 267.0
10		AGPA-13_1			299.0 / 148.0
11		AGPA-14			285.0 / 225.1
12		AGPA-14_1			285.0 / 213.1
13		Biochanin A			285.0 / 225.1 (2)
14		Biochanin A_1			285.0 / 213.0
15					

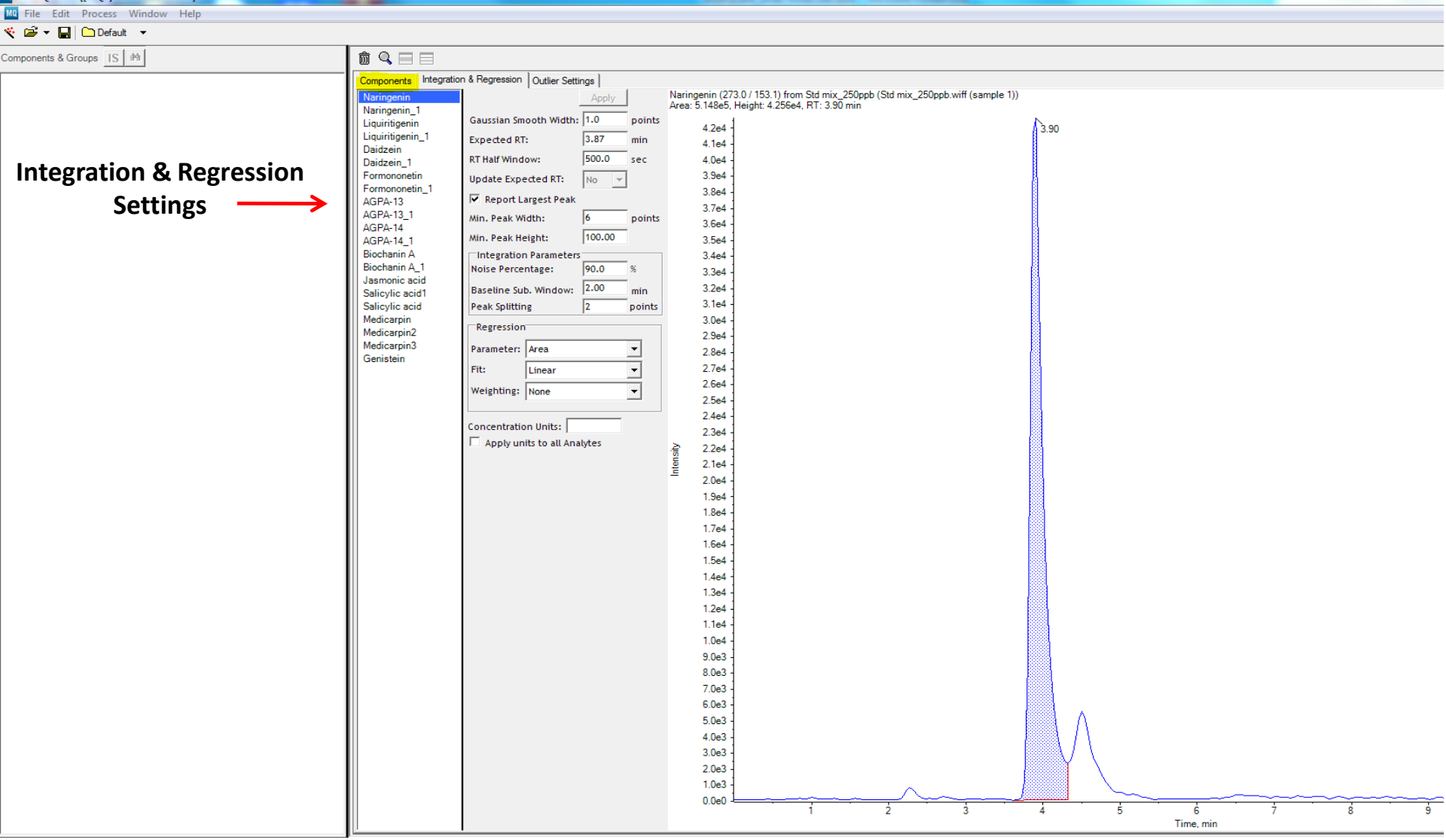
- You can also assign groups to the same set of molecules
- Check the IS box if any of the listed molecule was used as Internal standard

- Next go to Integration & Regression settings



Row	IS	Name	Group
1		Naringenin	
2		Naringenin_1	
3		Liquiritigenin	
4		Liquiritigenin_1	
5		Daidzein	
6		Daidzein_1	
7		Formononetin	
8		Formononetin_1	
9		AGPA-13	
10		AGPA-13_1	
11		AGPA-14	
12		AGPA-14_1	
13		Biochanin A	
14		Biochanin A_1	

Integration & Regression Settings →



- You can click on one component at a time and fill in the values for all the settings like smoothening, RT half window, Noise percentage etc.
- All these values will depend on the peak properties.
- Regression settings are used for plotting the calibration curve.
- Fill in the concentration units (eg. ng/ml, ppm etc.). Depends on the concentration of the standards prepared.

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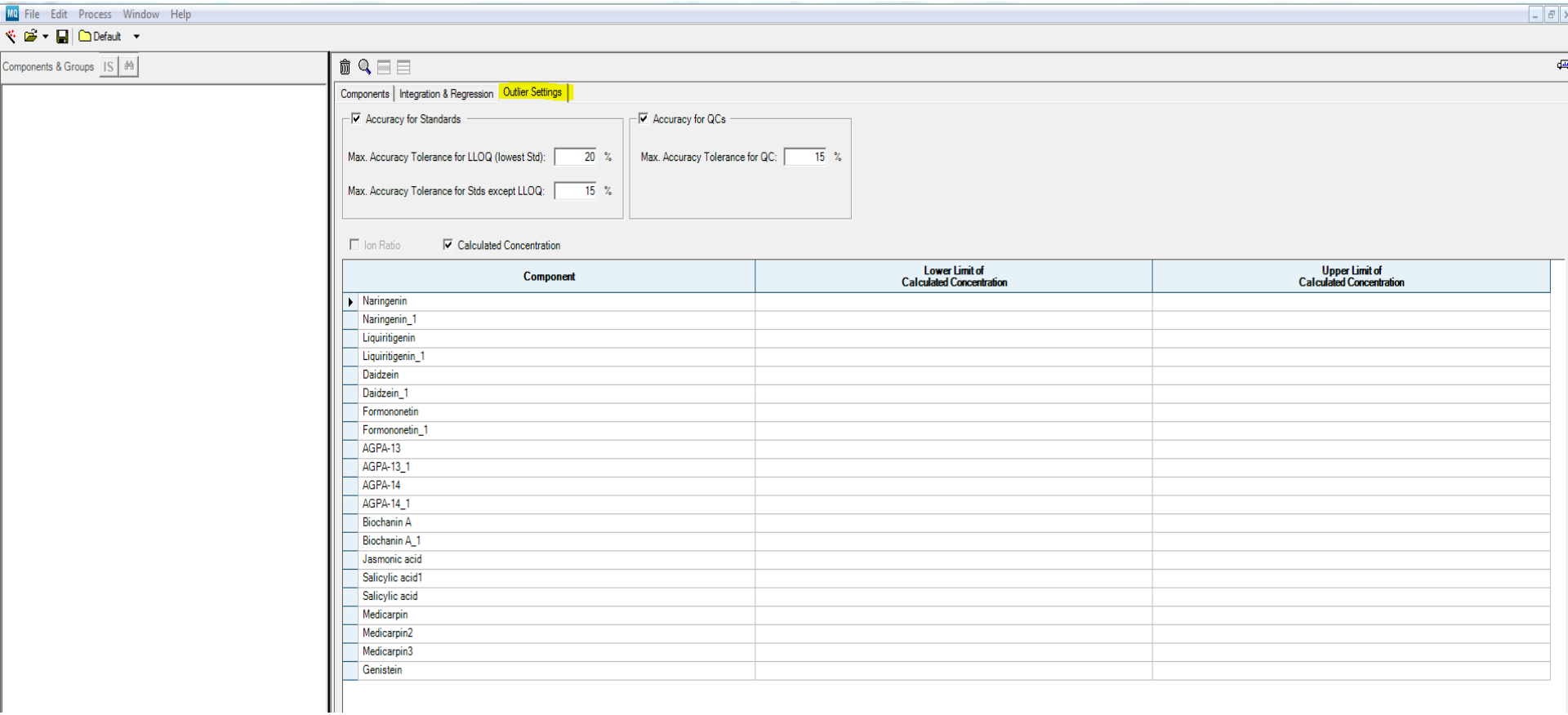
Outlier settings



Outlier settings are used if you need your results within a certain range like in Quality control

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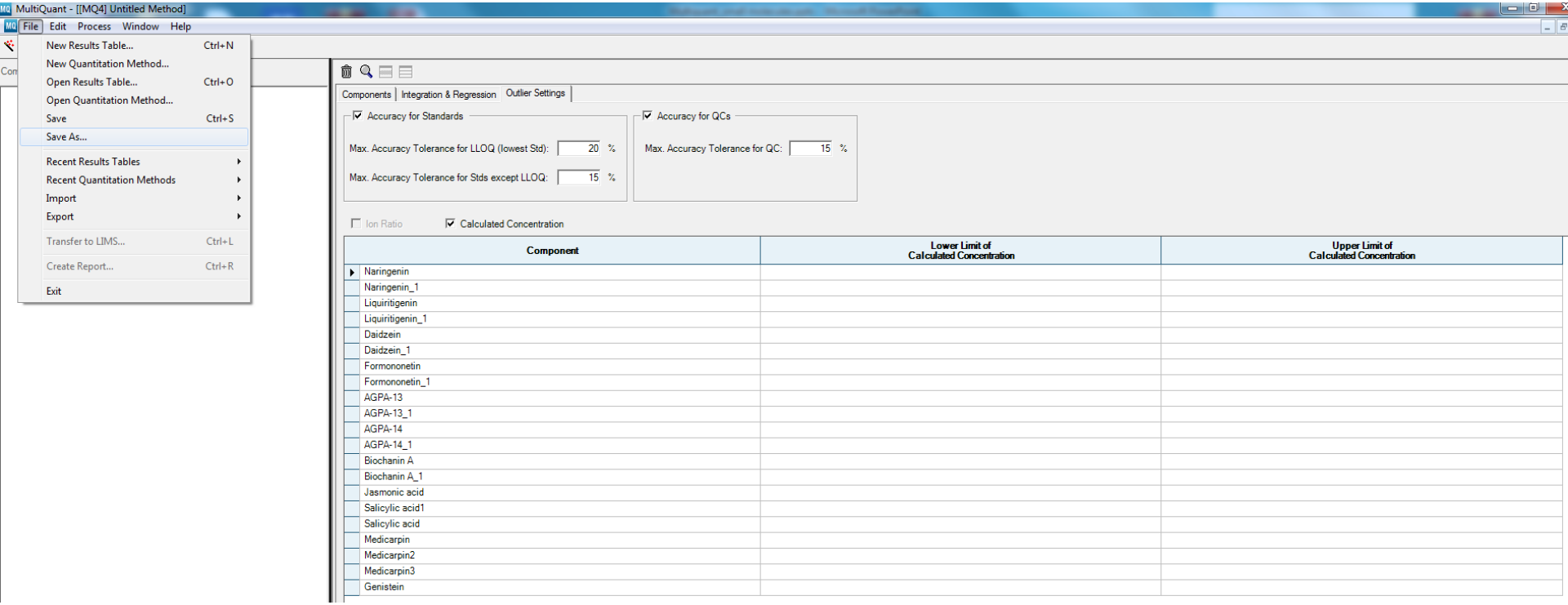
Save method;

Go to File> Save As> Give name to your method



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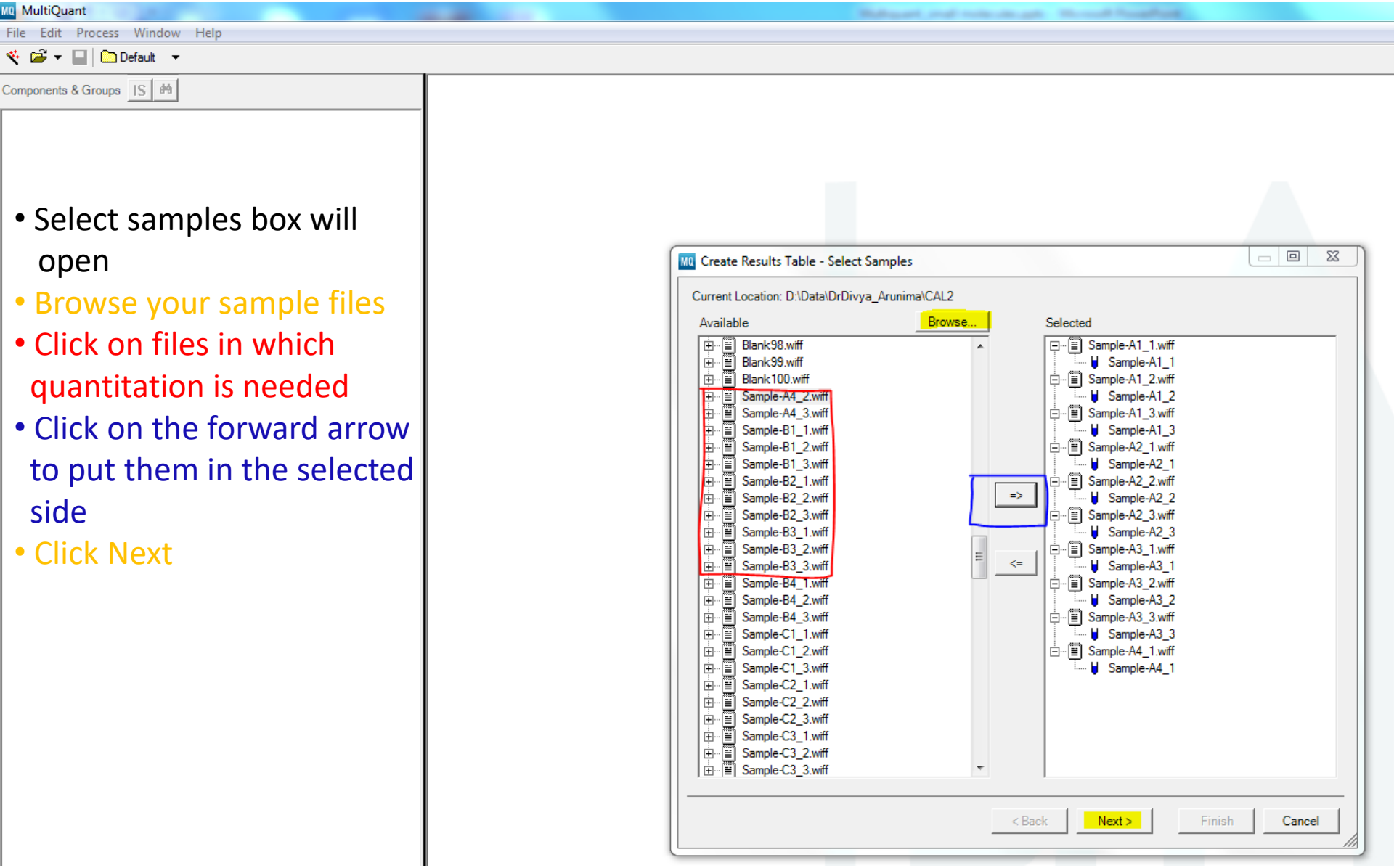
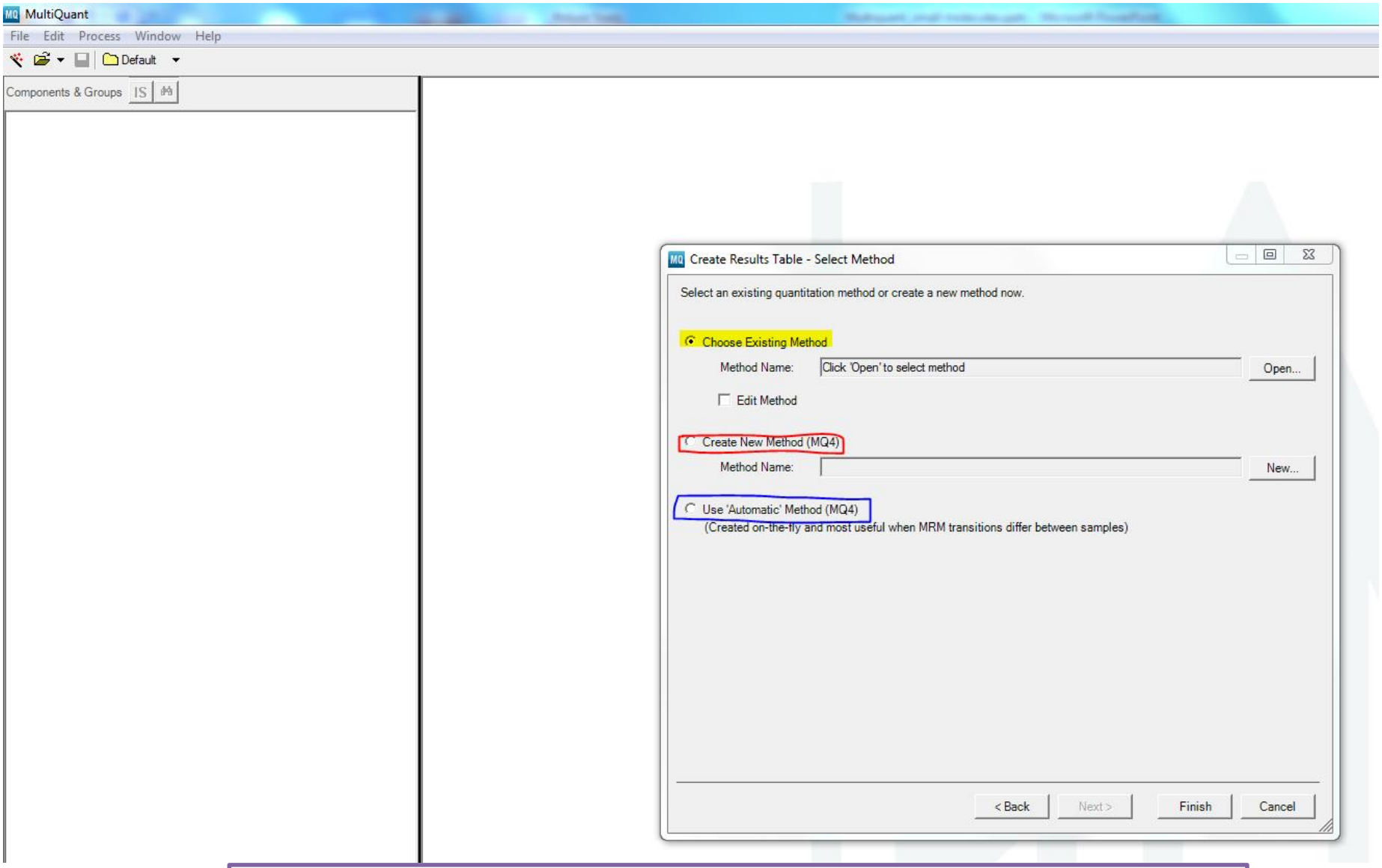
Creating Results Table:

Go to File> New Results table>

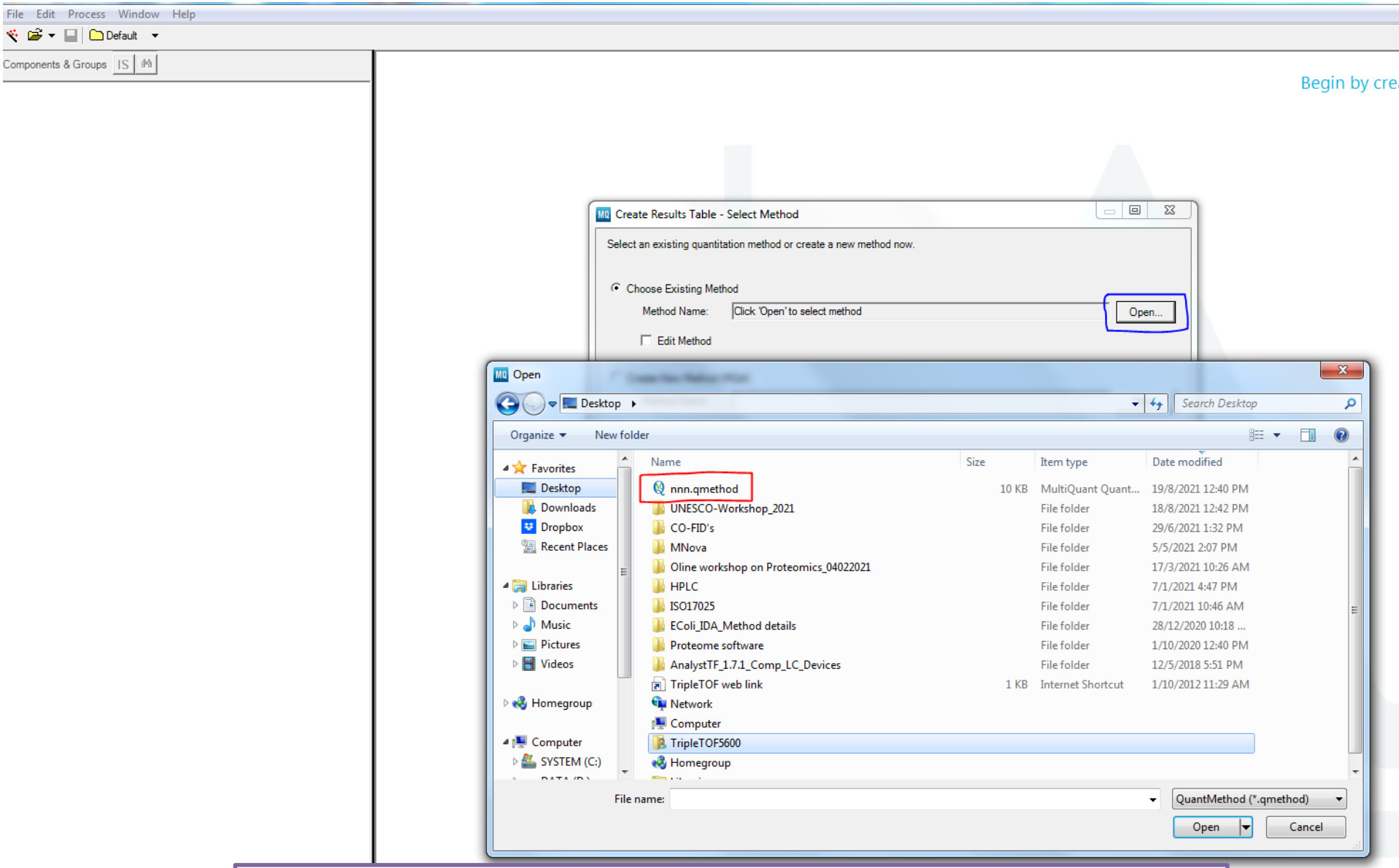


- Select samples box will open
- Browse your sample files
- Click on files in which quantitation is needed
- Click on the forward arrow to put them in the selected side
- Click Next

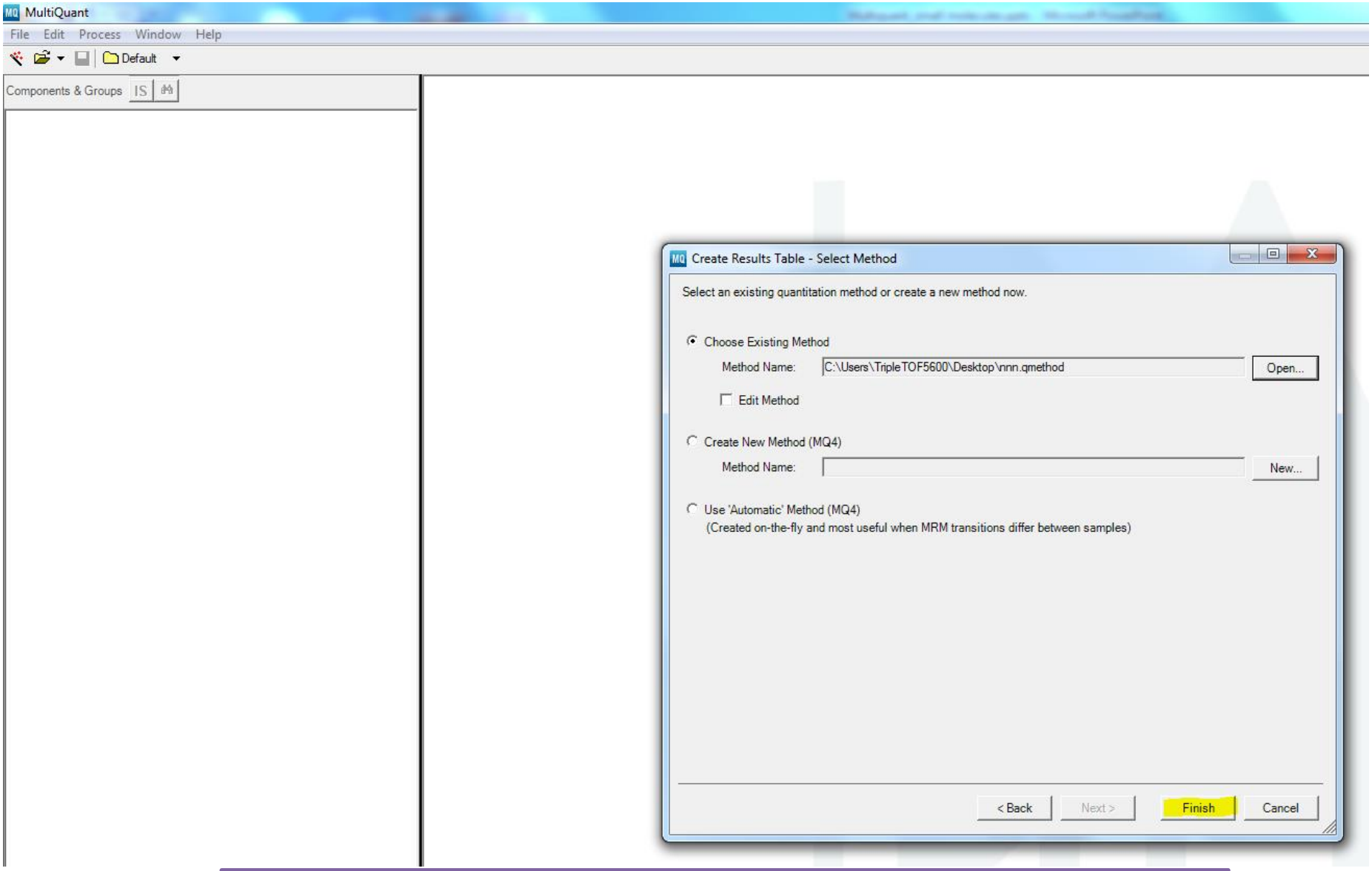
- Choose the method already created to process your sample files
- Check the “Create New Method” if the method was not created and saved before
- Automatic methods are generally experiment specific. In case of Lipids we can use it



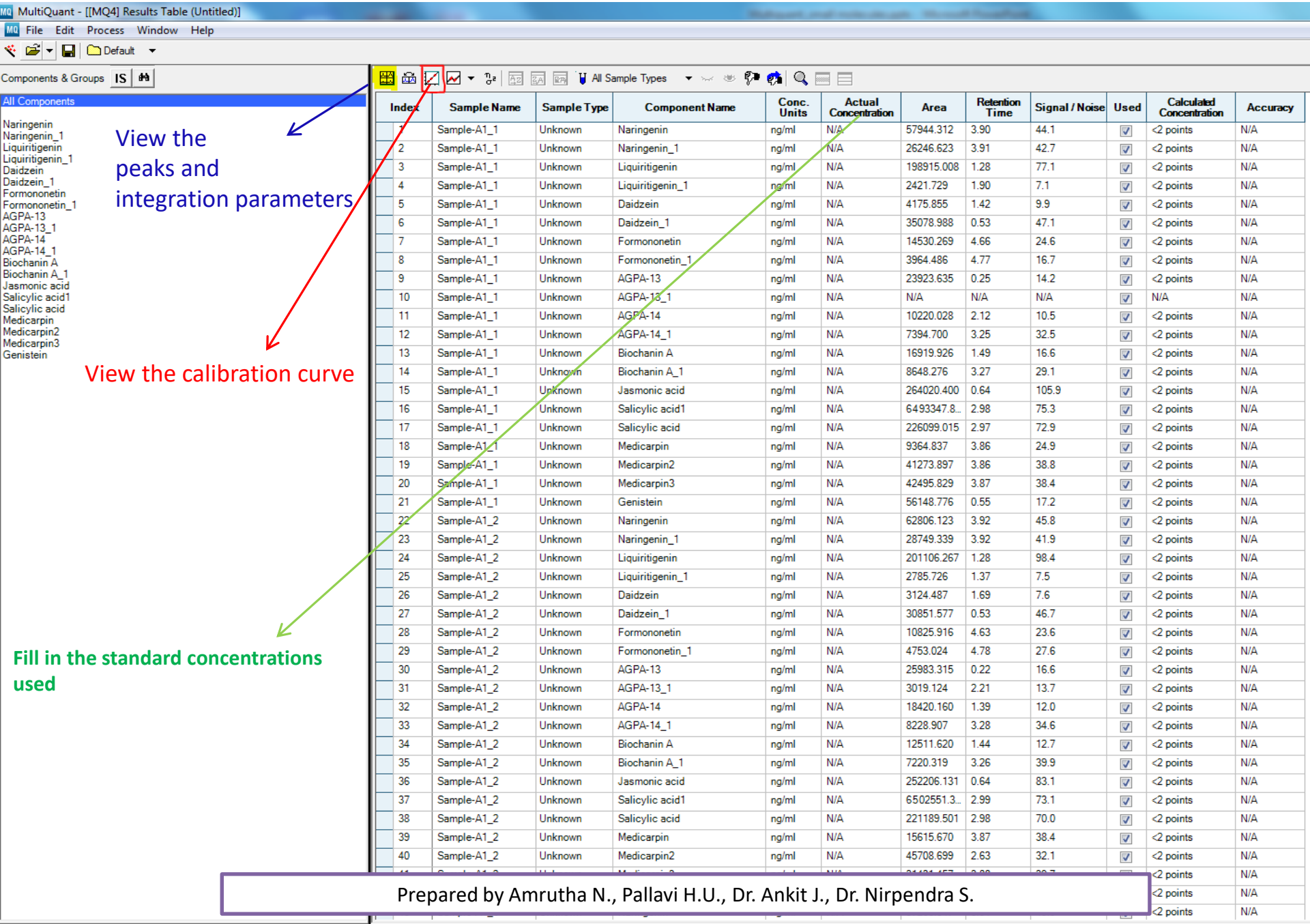
- Click " Open" to browse the saved method
- Method saved will have .qmethod as extension



- Click “Finish”



Results Table



Index	Sample Name	Sample Type	Component Name	Conc. Units	Actual Concentration	Area	Retention Time	Signal / Noise	Used	Calculated Concentration	Accuracy
1	Sample-A1_1	Unknown	Naringenin	ng/ml	N/A	57944.312	3.90	44.1	☑	<2 points	N/A
2	Sample-A1_1	Unknown	Naringenin_1	ng/ml	N/A	26246.623	3.91	42.7	☑	<2 points	N/A
3	Sample-A1_1	Unknown	Liquiritigenin	ng/ml	N/A	198915.008	1.28	77.1	☑	<2 points	N/A
4	Sample-A1_1	Unknown	Liquiritigenin_1	ng/ml	N/A	2421.729	1.90	7.1	☑	<2 points	N/A
5	Sample-A1_1	Unknown	Daidzein	ng/ml	N/A	4175.855	1.42	9.9	☑	<2 points	N/A
6	Sample-A1_1	Unknown	Daidzein_1	ng/ml	N/A	35078.988	0.53	47.1	☑	<2 points	N/A
7	Sample-A1_1	Unknown	Formononetin	ng/ml	N/A	14530.269	4.66	24.6	☑	<2 points	N/A
8	Sample-A1_1	Unknown	Formononetin_1	ng/ml	N/A	3964.486	4.77	16.7	☑	<2 points	N/A
9	Sample-A1_1	Unknown	AGPA-13	ng/ml	N/A	23923.635	0.25	14.2	☑	<2 points	N/A
10	Sample-A1_1	Unknown	AGPA-13_1	ng/ml	N/A	N/A	N/A	N/A	☑	N/A	N/A
11	Sample-A1_1	Unknown	AGPA-14	ng/ml	N/A	10220.028	2.12	10.5	☑	<2 points	N/A
12	Sample-A1_1	Unknown	AGPA-14_1	ng/ml	N/A	7394.700	3.25	32.5	☑	<2 points	N/A
13	Sample-A1_1	Unknown	Biochanin A	ng/ml	N/A	16919.926	1.49	16.6	☑	<2 points	N/A
14	Sample-A1_1	Unknown	Biochanin A_1	ng/ml	N/A	8648.276	3.27	29.1	☑	<2 points	N/A
15	Sample-A1_1	Unknown	Jasmonic acid	ng/ml	N/A	264020.400	0.64	105.9	☑	<2 points	N/A
16	Sample-A1_1	Unknown	Salicylic acid1	ng/ml	N/A	6493347.8...	2.98	75.3	☑	<2 points	N/A
17	Sample-A1_1	Unknown	Salicylic acid	ng/ml	N/A	226099.015	2.97	72.9	☑	<2 points	N/A
18	Sample-A1_1	Unknown	Medicarpin	ng/ml	N/A	9364.837	3.86	24.9	☑	<2 points	N/A
19	Sample-A1_1	Unknown	Medicarpin2	ng/ml	N/A	41273.897	3.86	38.8	☑	<2 points	N/A
20	Sample-A1_1	Unknown	Medicarpin3	ng/ml	N/A	42495.829	3.87	38.4	☑	<2 points	N/A
21	Sample-A1_1	Unknown	Genistein	ng/ml	N/A	56148.776	0.55	17.2	☑	<2 points	N/A
22	Sample-A1_2	Unknown	Naringenin	ng/ml	N/A	62806.123	3.92	45.8	☑	<2 points	N/A
23	Sample-A1_2	Unknown	Naringenin_1	ng/ml	N/A	28749.339	3.92	41.9	☑	<2 points	N/A
24	Sample-A1_2	Unknown	Liquiritigenin	ng/ml	N/A	201106.267	1.28	98.4	☑	<2 points	N/A
25	Sample-A1_2	Unknown	Liquiritigenin_1	ng/ml	N/A	2785.726	1.37	7.5	☑	<2 points	N/A
26	Sample-A1_2	Unknown	Daidzein	ng/ml	N/A	3124.487	1.69	7.6	☑	<2 points	N/A
27	Sample-A1_2	Unknown	Daidzein_1	ng/ml	N/A	30851.577	0.53	46.7	☑	<2 points	N/A
28	Sample-A1_2	Unknown	Formononetin	ng/ml	N/A	10825.916	4.63	23.6	☑	<2 points	N/A
29	Sample-A1_2	Unknown	Formononetin_1	ng/ml	N/A	4753.024	4.78	27.6	☑	<2 points	N/A
30	Sample-A1_2	Unknown	AGPA-13	ng/ml	N/A	25983.315	0.22	16.6	☑	<2 points	N/A
31	Sample-A1_2	Unknown	AGPA-13_1	ng/ml	N/A	3019.124	2.21	13.7	☑	<2 points	N/A
32	Sample-A1_2	Unknown	AGPA-14	ng/ml	N/A	18420.160	1.39	12.0	☑	<2 points	N/A
33	Sample-A1_2	Unknown	AGPA-14_1	ng/ml	N/A	8228.907	3.28	34.6	☑	<2 points	N/A
34	Sample-A1_2	Unknown	Biochanin A	ng/ml	N/A	12511.620	1.44	12.7	☑	<2 points	N/A
35	Sample-A1_2	Unknown	Biochanin A_1	ng/ml	N/A	7220.319	3.26	39.9	☑	<2 points	N/A
36	Sample-A1_2	Unknown	Jasmonic acid	ng/ml	N/A	252206.131	0.64	83.1	☑	<2 points	N/A
37	Sample-A1_2	Unknown	Salicylic acid1	ng/ml	N/A	6502551.3...	2.99	73.1	☑	<2 points	N/A
38	Sample-A1_2	Unknown	Salicylic acid	ng/ml	N/A	221189.501	2.98	70.0	☑	<2 points	N/A
39	Sample-A1_2	Unknown	Medicarpin	ng/ml	N/A	15615.670	3.87	38.4	☑	<2 points	N/A
40	Sample-A1_2	Unknown	Medicarpin2	ng/ml	N/A	45708.699	2.63	32.1	☑	<2 points	N/A
										<2 points	N/A
										<2 points	N/A
										<2 points	N/A

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File Edit Process Window Help

Default

Components & Groups | IS

All Components

Naringenin
Naringenin_1
Liquiritigenin
Liquiritigenin_1
Daidzein
Daidzein_1
Formononetin
Formononetin_1
AGPA-13
AGPA-13_1
AGPA-14
AGPA-14_1
Biochanin A
Biochanin A_1
Jasmonic acid
Salicylic acid1
Salicylic acid
Medicarpin
Medicarpin2
Medicarpin3
Genistein

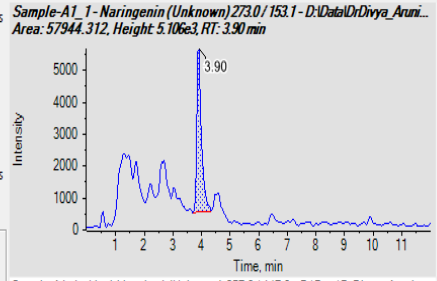
All Sample Types

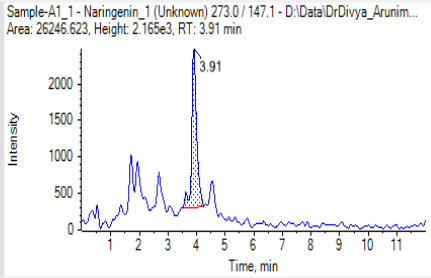
Index	Sample Name	Sample Type	Component Name	Conc. Units	Actual Concentration	Area	Retention Time	Signal / Noise	Used	Calculated Concentration	Accuracy
1	Sample-A1_1	Unknown	Naringenin	ng/ml	N/A	57944.312	3.90	44.1	☑	<2 points	N/A
2	Sample-A1_1	Unknown	Naringenin_1	ng/ml	N/A	26246.623	3.91	42.7	☑	<2 points	N/A
3	Sample-A1_1	Unknown	Liquiritigenin	ng/ml	N/A	198915.008	1.28	77.1	☑	<2 points	N/A
4	Sample-A1_1	Unknown	Liquiritigenin_1	ng/ml	N/A	2421.729	1.90	7.1	☑	<2 points	N/A
5	Sample-A1_1	Unknown	Daidzein	ng/ml	N/A	4175.855	1.42	9.9	☑	<2 points	N/A
6	Sample-A1_1	Unknown	Daidzein_1	ng/ml	N/A	35078.988	0.53	47.1	☑	<2 points	N/A
7	Sample-A1_1	Unknown	Formononetin	ng/ml	N/A	14530.269	4.66	24.6	☑	<2 points	N/A
8	Sample-A1_1	Unknown	Formononetin_1	ng/ml	N/A	3964.486	4.77	16.7	☑	<2 points	N/A
9	Sample-A1_1	Unknown	AGPA-13	ng/ml	N/A	23923.635	0.25	14.2	☑	<2 points	N/A
10	Sample-A1_1	Unknown	AGPA-13_1	ng/ml	N/A	N/A	N/A	N/A	☑	N/A	N/A
11	Sample-A1_1	Unknown	AGPA-14	ng/ml	N/A	10220.028	2.12	10.5	☑	<2 points	N/A
12	Sample-A1_1	Unknown	AGPA-14_1	ng/ml	N/A	7394.700	3.25	32.5	☑	<2 points	N/A
13	Sample-A1_1	Unknown	Biochanin A	ng/ml	N/A	16919.926	1.49	16.6	☑	<2 points	N/A
14	Sample-A1_1	Unknown	Biochanin A_1	ng/ml	N/A	8648.276	3.27	29.1	☑	<2 points	N/A
15	Sample-A1_1	Unknown	Jasmonic acid	ng/ml	N/A	264020.400	0.64	105.9	☑	<2 points	N/A
16	Sample-A1_1	Unknown	Salicylic acid1	ng/ml	N/A	6493347.8..	2.98	75.3	☑	<2 points	N/A
17	Sample-A1_1	Unknown	Salicylic acid	ng/ml	N/A	226099.015	2.97	72.9	☑	<2 points	N/A
18	Sample-A1_1	Unknown	Medicarpin	ng/ml	N/A	9364.837	3.86	24.9	☑	<2 points	N/A
19	Sample-A1_1	Unknown	Medicarpin2	ng/ml	N/A	41273.897	3.86	38.8	☑	<2 points	N/A
20	Sample-A1_1	Unknown	Medicarpin3	ng/ml	N/A	42495.829	3.87	38.4	☑	<2 points	N/A

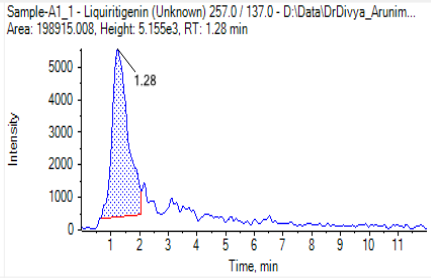
Apply | Manual Integration

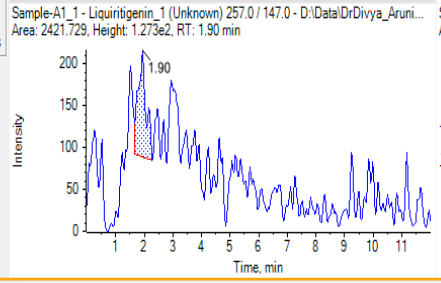
Gaussian Smooth Width: 1.0 points
Expected RT: 3.87 min
RT Half Window: 500.0 sec
Update Expected RT: No
☑ Report Largest Peak
Min. Peak Width: 6 points
Min. Peak Height: 100.00

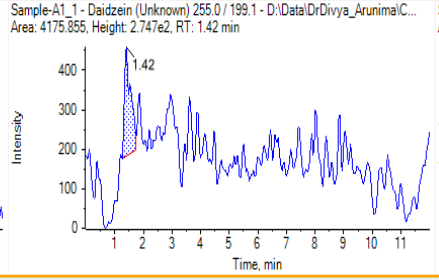
Integration Parameters
Noise Percentage: 90.0 %
Baseline Sub. Window: 2.00 min
Peak Splitting: 2 points

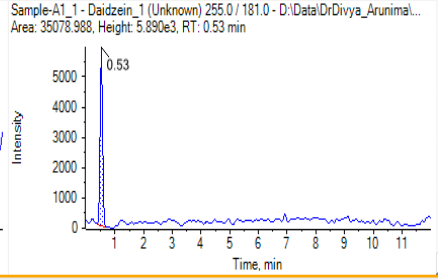












Peak Review Window

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File Edit Process Window Help

Default

Components & Groups | IS

All Components

Naringenin
Liquiritigenin
Daidzein
Formononetin
2'-methoxyformononetin
2-hydroxyformononetin
Biochanin A
Jasmonic acid
Salicylic acid
Medicarpin
Genistein

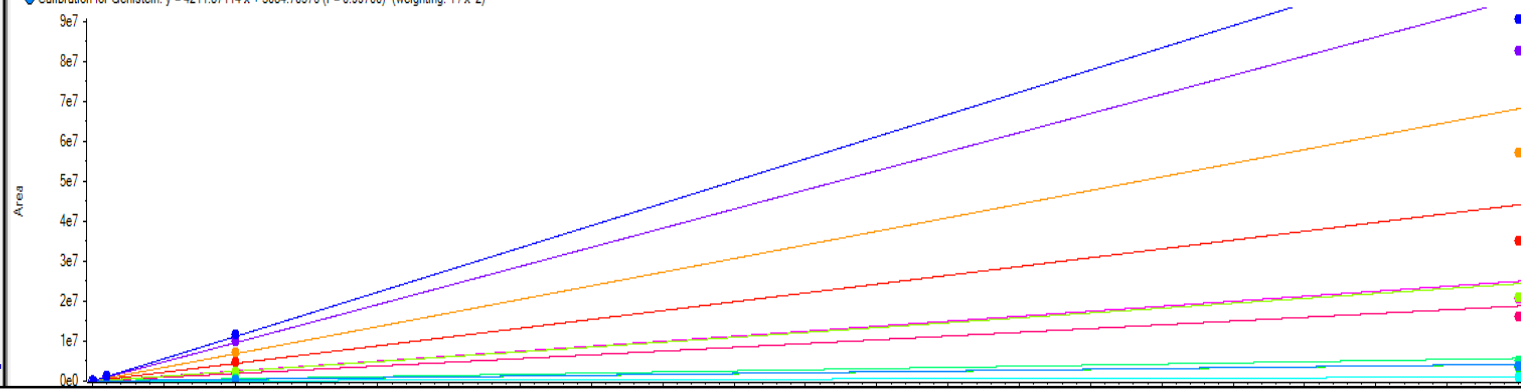
All Sample Types

Index	Sample Name	Sample Type	Component Name	Conc. Units	Actual Concentration	Area	Retention Time	Signal / Noise	Used	Calculated Concentration	Accuracy
1	std mix_0.1ppb_1-10	Standard	Naringenin	ng/ml	0.10	25835.882	3.86	29.2	☐	< 0	N/A
2	std mix_0.1ppb_1-10	Standard	Liquiritigenin	ng/ml	0.10	23407.713	3.02	28.0	☐	< 0	N/A
3	std mix_0.1ppb_1-10	Standard	Daidzein	ng/ml	0.10	4586.277	2.85	5.7	☐	< 0	N/A
4	std mix_0.1ppb_1-10	Standard	Formononetin	ng/ml	0.10	5141.946	4.79	19.1	☐	< 0	N/A
5	std mix_0.1ppb_1-10	Standard	2'-methoxyformononetin	ng/ml	0.10	14693.126	4.57	64.9	☐	< 0	N/A
6	std mix_0.1ppb_1-10	Standard	2'-hydroxyformononetin	ng/ml	0.10	13196.485	4.10	18.2	☐	< 0	N/A
7	std mix_0.1ppb_1-10	Standard	Biochanin A	ng/ml	0.10	3981.709	5.97	17.9	☐	< 0	N/A
8	std mix_0.1ppb_1-10	Standard	Jasmonic acid	ng/ml	0.10	1257.010	4.12	13.1	☐	< 0	N/A
9	std mix_0.1ppb_1-10	Standard	Salicylic acid	ng/ml	0.10	3607.749	3.04	5.3	☐	0.271	271.34
10	std mix_0.1ppb_1-10	Standard	Medicarpin	ng/ml	0.10	623.196	3.63	6.7	☐	< 0	N/A
11	std mix_0.1ppb_1-10	Standard	Genistein	ng/ml	0.10	1055.112	4.32	4.0	☐	< 0	N/A
12	std mix_1ppb_1-10	Standard	Naringenin	ng/ml	1.00	145818.093	3.90	125.8	☑	0.984	98.45
13	std mix_1ppb_1-10	Standard	Liquiritigenin	ng/ml	1.00	132020.070	3.09	110.8	☑	0.988	98.82
14	std mix_1ppb_1-10	Standard	Daidzein	ng/ml	1.00	37247.573	2.94	48.5	☑	0.982	98.20
15	std mix_1ppb_1-10	Standard	Formononetin	ng/ml	1.00	32696.654	4.79	92.9	☑	0.987	98.73
16	std mix_1ppb_1-10	Standard	2'-methoxyformononetin	ng/ml	1.00	65272.568	4.59	331.5	☑	0.982	98.22
17	std mix_1ppb_1-10	Standard	2'-hydroxyformononetin	ng/ml	1.00	98725.165	4.13	93.1	☑	0.988	98.84
18	std mix_1ppb_1-10	Standard	Biochanin A	ng/ml	1.00	34840.550	5.98	81.9	☑	0.986	98.61
19	std mix_1ppb_1-10	Standard	Jasmonic acid	ng/ml	1.00	6345.081	4.16	35.4	☑	0.989	98.94
20	std mix_1ppb_1-10	Standard	Salicylic acid	ng/ml	1.00	7721.918	2.99	17.3	☑	0.992	99.24

Any deviating concentration value can be excluded

Regression

Calibration for Naringenin: y = 1.11106e5 x + 3.64389e4 (r = 0.98936) (weighting: 1 / x^2)
Calibration for Liquiritigenin: y = 9.55825e4 x + 3.75625e4 (r = 0.99408) (weighting: 1 / x^2)
Calibration for Daidzein: y = 24964.18578 x + 12731.99781 (r = 0.98896) (weighting: 1 / x^2)
Calibration for Formononetin: y = 18768.74886 x + 14167.16404 (r = 0.99321) (weighting: 1 / x^2)
Calibration for 2'-methoxyformononetin: y = 4.40886e4 x + 21968.36246 (r = 0.98650) (weighting: 1 / x^2)
Calibration for 2'-hydroxyformononetin: y = 6.80936e4 x + 3.14221e4 (r = 0.99267) (weighting: 1 / x^2)
Calibration for Biochanin A: y = 24358.59612 x + 10821.53068 (r = 0.99323) (weighting: 1 / x^2)
Calibration for Jasmonic acid: y = 4179.64080 x + 2209.77387 (r = 0.99428) (weighting: 1 / x^2)
Calibration for Salicylic acid: y = 5705.55979 x + 2059.60138 (r = 0.99659) (weighting: 1 / x^2)
Calibration for Medicarpin: y = 993.90006 x + 990.26851 (r = 0.99363) (weighting: 1 / x^2)
Calibration for Genistein: y = 4211.87114 x + 3084.70576 (r = 0.99700) (weighting: 1 / x^2)



Cal Curves for all Components
- Regression equation
- Co-relation coefficient

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