MICROMASS

Operational Qualification for the QuanLynx Application of MassLynx V4.0

1. Contents

2. Purpose

This document describes a series of steps that when performed correctly will provide a means to ensure that the QuanLynx Application Manager is working as expected. Included in the Appendix are details of the actual algorithms employed by the application to calculate the results generated.

3. Scope

This document may be used by any QuanLynx Application Manager user to perform acceptance testing of the Application. It may also be used to derive user defined acceptance testing.

4. Revision History

This is the first issue of this document for the MassLynx Version 4.0 software.

5. Method

Using the standard data set provided with the MassLynx installation follow the steps detailed in the Test Preparation and Test Procedure sections, signing off each test that conforms to the expected results. On completion of the test the results should then be compared to the Acceptance Criteria and an overall assessment made on these results.

6. Acceptance Criteria

The test results must exactly match the results shown in this document unless otherwise indicated. The display appearance may not be identical to that shown in this document, as this will depend on the version of Windows being used and its configuration. In particular the Calibration Window header will differ from that shown in this document.

7. Results

A test result may be any one or combination of the following.

- data produced during the test which is saved to disk.
- printed data produced during the test and filed with the Operational Qualification.
- an action occurring during the test which is observed by the tester.

8. Test Preparation

Install MassLynx on a suitable computer. Depending on the screen resolution selected on the PC the screen may look slightly different to those shown in this document, these should only be minor differences and will not affect the test results. Check that the project QUANTIFY.PRO is also available on the system.
To ensure that a known starting point for the test to be carried out the QuanLynx default settings need to be restored. From the MassLynx directory delete the file quanLynx.~ql, this will delete the current format settings and returns these to the default values.
Start MassLynx as normal responding to any prompts until the MassLynx top level browser window appears. If Security is installed a MassLynx Administrator should ensure the User account used has the privileges required to complete this test.

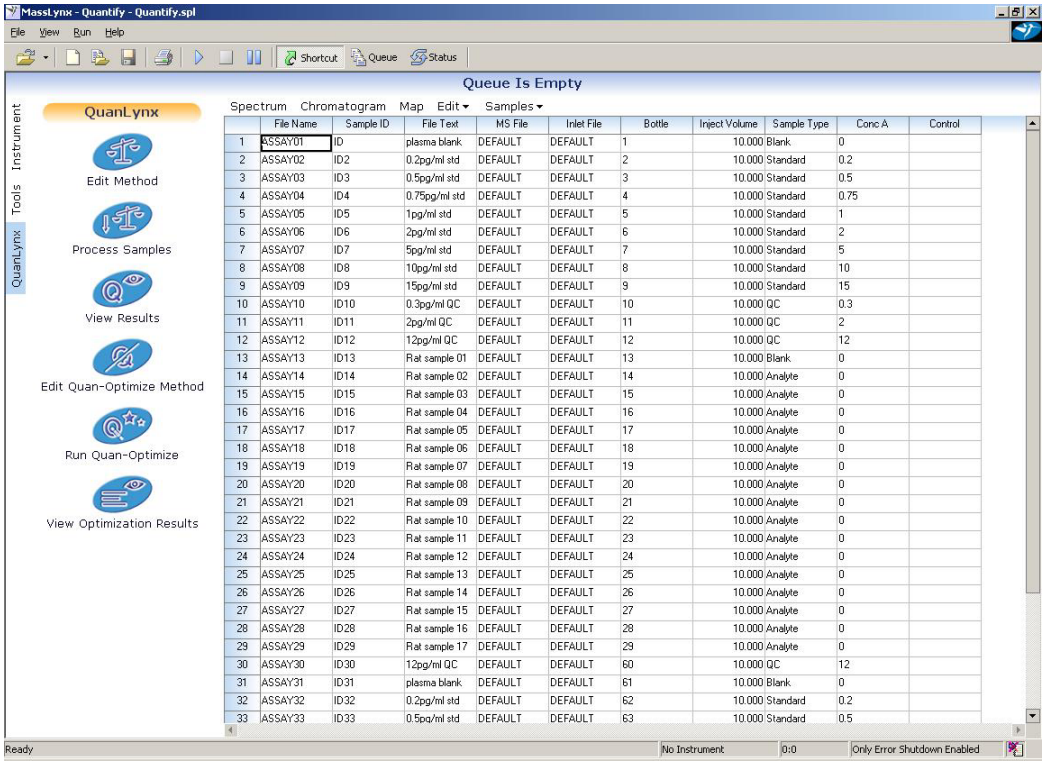
Checked _____ Date _____

9. Test Procedure

9.1 Project Selection

From the MassLynx main menu select **File|Open Project…** Choose the quantify.pro project that can be found in the MassLynx directory and click **OK**. Now from the **Sample List** menu **Samples** select **Format|Load…** and select quantify.fmt and click **OK**. Select the **QuanLynx** shortcuts.

The Sample List should look like the one shown in Figure 1 below.



	File Name	Sample ID	File Text	MS File	Inlet File	Bottle	Inject Volume	Sample Type	Conc A	Control
1	ASSAY01	ID	plasma blank	DEFAULT	DEFAULT	1	10.000	Blank	0	
2	ASSAY02	ID2	0.2pg/ml std	DEFAULT	DEFAULT	2	10.000	Standard	0.2	
3	ASSAY03	ID3	0.5pg/ml std	DEFAULT	DEFAULT	3	10.000	Standard	0.5	
4	ASSAY04	ID4	0.75pg/ml std	DEFAULT	DEFAULT	4	10.000	Standard	0.75	
5	ASSAY05	ID5	1pg/ml std	DEFAULT	DEFAULT	5	10.000	Standard	1	
6	ASSAY06	ID6	2pg/ml std	DEFAULT	DEFAULT	6	10.000	Standard	2	
7	ASSAY07	ID7	5pg/ml std	DEFAULT	DEFAULT	7	10.000	Standard	5	
8	ASSAY08	ID8	10pg/ml std	DEFAULT	DEFAULT	8	10.000	Standard	10	
9	ASSAY09	ID9	15pg/ml std	DEFAULT	DEFAULT	9	10.000	Standard	15	
10	ASSAY10	ID10	0.3pg/ml QC	DEFAULT	DEFAULT	10	10.000	QC	0.3	
11	ASSAY11	ID11	2pg/ml QC	DEFAULT	DEFAULT	11	10.000	QC	2	
12	ASSAY12	ID12	12pg/ml QC	DEFAULT	DEFAULT	12	10.000	QC	12	
13	ASSAY13	ID13	Rat sample 01	DEFAULT	DEFAULT	13	10.000	Blank	0	
14	ASSAY14	ID14	Rat sample 02	DEFAULT	DEFAULT	14	10.000	Analyte	0	
15	ASSAY15	ID15	Rat sample 03	DEFAULT	DEFAULT	15	10.000	Analyte	0	
16	ASSAY16	ID16	Rat sample 04	DEFAULT	DEFAULT	16	10.000	Analyte	0	
17	ASSAY17	ID17	Rat sample 05	DEFAULT	DEFAULT	17	10.000	Analyte	0	
18	ASSAY18	ID18	Rat sample 06	DEFAULT	DEFAULT	18	10.000	Analyte	0	
19	ASSAY19	ID19	Rat sample 07	DEFAULT	DEFAULT	19	10.000	Analyte	0	
20	ASSAY20	ID20	Rat sample 08	DEFAULT	DEFAULT	20	10.000	Analyte	0	
21	ASSAY21	ID21	Rat sample 09	DEFAULT	DEFAULT	21	10.000	Analyte	0	
22	ASSAY22	ID22	Rat sample 10	DEFAULT	DEFAULT	22	10.000	Analyte	0	
23	ASSAY23	ID23	Rat sample 11	DEFAULT	DEFAULT	23	10.000	Analyte	0	
24	ASSAY24	ID24	Rat sample 12	DEFAULT	DEFAULT	24	10.000	Analyte	0	
25	ASSAY25	ID25	Rat sample 13	DEFAULT	DEFAULT	25	10.000	Analyte	0	
26	ASSAY26	ID26	Rat sample 14	DEFAULT	DEFAULT	26	10.000	Analyte	0	
27	ASSAY27	ID27	Rat sample 15	DEFAULT	DEFAULT	27	10.000	Analyte	0	
28	ASSAY28	ID28	Rat sample 16	DEFAULT	DEFAULT	28	10.000	Analyte	0	
29	ASSAY29	ID29	Rat sample 17	DEFAULT	DEFAULT	29	10.000	Analyte	0	
30	ASSAY30	ID30	12pg/ml QC	DEFAULT	DEFAULT	60	10.000	QC	12	
31	ASSAY31	ID31	plasma blank	DEFAULT	DEFAULT	61	10.000	Blank	0	
32	ASSAY32	ID32	0.2pg/ml std	DEFAULT	DEFAULT	62	10.000	Standard	0.2	
33	ASSAY33	ID33	0.5pg/ml std	DEFAULT	DEFAULT	63	10.000	Standard	0.5	

Figure 1 Quantify.pro sample list.

Note The actual items that appear on the **QuanLynx** shortcut will depend on the chosen installation options.

Checked _____ Date _____

9.2 Import File

From the MassLynx top level with **QuanLynx** shortcuts selected, click **View Results** to open the QuanLynx Browser. From the **File** menu select **Import Quan Data…** Select the project.ini file from the quantify.pro folder found in the MassLynx directory and click **OK**. Whilst the project is being imported a progress bar will be displayed at the bottom right of the Browser window.
The QuanLynx Browser should now look similar to that shown in Figure 2 below.

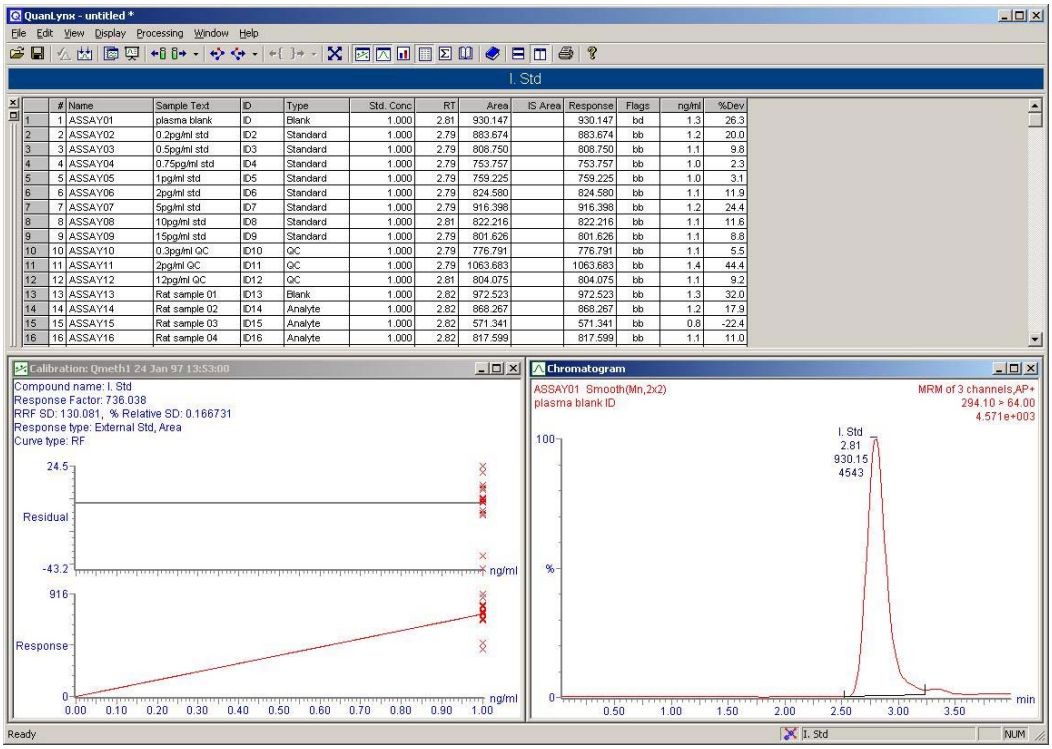


Figure 2 Data import in QuanLynx

Note The window layout may differ if these have been altered since MassLynx was installed.

Checked _____ Date _____

9.3 Initial Data Values

In the QuanLynx Browser ensure that **1. Std** is selected and check that the Results Summary contains the data as shown in Table 1 below.

#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
1	ASSAY01	plasma blank	ID	Blank	1.000	2.81	930.147		930.147	bd	1.3	26.3
2	ASSAY02	0.2pg/ml std	ID2	Standard	1.000	2.79	883.674		883.674	bb	1.2	20.0
3	ASSAY03	0.5pg/ml std	ID3	Standard	1.000	2.79	808.750		808.750	bb	1.1	9.8
4	ASSAY04	0.75pg/ml std	ID4	Standard	1.000	2.79	753.757		753.757	bb	1.0	2.3
5	ASSAY05	1pg/ml std	ID5	Standard	1.000	2.79	759.225		759.225	bb	1.0	3.1
6	ASSAY06	2pg/ml std	ID6	Standard	1.000	2.79	824.580		824.580	bb	1.1	11.9
7	ASSAY07	5pg/ml std	ID7	Standard	1.000	2.79	916.398		916.398	bb	1.2	24.4
8	ASSAY08	10pg/ml std	ID8	Standard	1.000	2.81	822.216		822.216	bb	1.1	11.6
9	ASSAY09	15pg/ml std	ID9	Standard	1.000	2.79	801.626		801.626	bb	1.1	8.8
10	ASSAY10	0.3pg/ml QC	ID10	QC	1.000	2.79	776.791		776.791	bb	1.1	5.5
11	ASSAY11	2pg/ml QC	ID11	QC	1.000	2.79	1063.683		1063.683	bb	1.4	44.4
12	ASSAY12	12pg/ml QC	ID12	QC	1.000	2.81	804.075		804.075	bb	1.1	9.2
13	ASSAY13	Rat sample 01	ID13	Blank	1.000	2.82	972.523		972.523	bb	1.3	32.0
14	ASSAY14	Rat sample 02	ID14	Analyte	1.000	2.82	868.267		868.267	bb	1.2	17.9
15	ASSAY15	Rat sample 03	ID15	Analyte	1.000	2.82	571.341		571.341	bb	0.8	-22.4
16	ASSAY16	Rat sample 04	ID16	Analyte	1.000	2.82	817.599		817.599	bb	1.1	11.0
17	ASSAY17	Rat sample 05	ID17	Analyte	1.000	2.82	817.181		817.181	bb	1.1	10.9
18	ASSAY18	Rat sample 06	ID18	Analyte	1.000	2.82	727.503		727.503	bb	1.0	-1.2
19	ASSAY19	Rat sample 07	ID19	Analyte	1.000	2.82	924.266		924.266	bb	1.3	25.5
20	ASSAY20	Rat sample 08	ID20	Analyte	1.000	2.82	641.667		641.667	bb	0.9	-12.9
21	ASSAY21	Rat sample 09	ID21	Analyte	1.000	2.82	690.912		690.912	bb	0.9	-6.2
22	ASSAY22	Rat sample 10	ID22	Analyte	1.000	2.82	692.057		692.057	bb	0.9	-6.0
23	ASSAY23	Rat sample 11	ID23	Analyte	1.000	2.82	890.020		890.020	bb	1.2	20.8
24	ASSAY24	Rat sample 12	ID24	Analyte	1.000	2.82	835.173		835.173	bb	1.1	13.4
25	ASSAY25	Rat sample 13	ID25	Analyte	1.000	2.82	372.693		372.693	bb	0.5	-49.4
26	ASSAY26	Rat sample 14	ID26	Analyte	1.000	2.82	816.763		816.763	bb	1.1	10.9
27	ASSAY27	Rat sample 15	ID27	Analyte	1.000	2.82	733.391		733.391	bb	1.0	-0.4
28	ASSAY28	Ratsample 16	ID28	Analyte	1.000	2.82	736.818		736.818	bb	1.0	0.0
29	ASSAY29	Rat sample 17	ID29	Analyte	1.000	2.84	782.950		782.950	bb	1.1	6.3
30	ASSAY30	12pg/ml QC	ID60	QC	1.000	2.81	1263.891		1263.891	bb	1.7	71.6
31	ASSAY31	plasma blank	ID61	Blank	1.000	2.82	13.485		13.485	bb	0.0	-98.2
32	ASSAY32	0.2pg/ml std	ID62	Standard	1.000	2.81	685.769		685.769	bb	0.9	-6.9
33	ASSAY33	0.5pg/ml std	ID63	Standard	1.000	2.81	683.362		683.362	bd	0.9	-7.2
34	ASSAY34	0.75pg/ml std	ID64	Standard	1.000	2.81	699.598		699.598	bb	0.9	-5.0
35	ASSAY35	1pg/ml std	ID65	Standard	1.000	2.81	417.738		417.738	bb	0.6	-43.3
36	ASSAY36	2pg/ml std	ID66	Standard	1.000	2.81	761.854		761.854	bd	1.0	3.4
37	ASSAY37	5pg/ml std	ID67	Standard	1.000	2.81	740.750		740.750	bb	1.0	0.6
38	ASSAY38	10pg/ml std	ID68	Standard	1.000	2.79	480.845		480.845	bb	0.7	-34.7
39	ASSAY39	15pg/ml std	ID69	Standard	1.000	2.81	745.369		745.369	bb	1.0	1.2

Table 1 Initial results summary

Checked _____ Date _____

9.4 Calculations

From the QuanLynx options select **Process Samples** and set the parameters as shown in Figure 3 below.

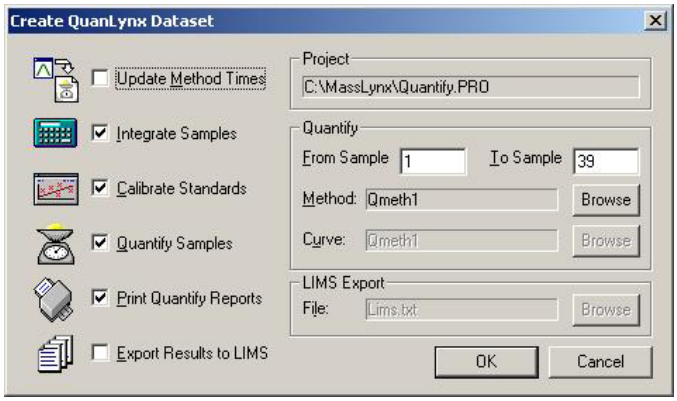


Figure 3 Create QuanLynx dataset

Click the **OK** button and then maximise the QuanLynx Browser. Click **Yes** to the prompt to Save changes to the untitled file. Then **Save As** test1.
Whilst the data is being processed a progress bar will be displayed at the bottom right of the Browser window.

Checked _____ Date _____

9.5 Results

In the QuanLynx Browser the results displayed will automatically be **1. Std**, which is an internal standard. Check that the results summary contains the data as shown in Table 2 below.

#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
1	ASSAY01	plasma blank	ID	Blank	1.000	2.81	930.147		930.147	bd	1.3	26.3
2	ASSAY02	0.2pg/ml std	ID2	Standard	1.000	2.79	883.674		883.674	bb	1.2	20.0
3	ASSAY03	0.5pg/ml std	ID3	Standard	1.000	2.79	808.750		808.750	bb	1.1	9.8
4	ASSAY04	0.75pg/ml std	ID4	Standard	1.000	2.79	753.757		753.757	bb	1.0	2.3
5	ASSAY05	1pg/ml std	ID5	Standard	1.000	2.79	759.225		759.225	bb	1.0	3.1
6	ASSAY06	2pg/ml std	ID6	Standard	1.000	2.79	824.580		824.580	bb	1.1	11.9
7	ASSAY07	5pg/ml std	ID7	Standard	1.000	2.79	916.398		916.398	bb	1.2	24.4
8	ASSAY08	10pg/ml std	ID8	Standard	1.000	2.81	822.216		822.216	bb	1.1	11.6
9	ASSAY09	15pg/ml std	ID9	Standard	1.000	2.79	801.626		801.626	bb	1.1	8.8
10	ASSAY10	0.3pg/ml QC	ID10	QC	1.000	2.79	776.791		776.791	bb	1.1	5.5
11	ASSAY11	2pg/ml QC	ID11	QC	1.000	2.79	1063.683		1063.683	bb	1.4	44.4
12	ASSAY12	12pg/ml QC	ID12	QC	1.000	2.81	804.075		804.075	bb	1.1	9.2
13	ASSAY13	Rat sample 01	ID13	Blank	1.000	2.82	972.523		972.523	bb	1.3	32.0
14	ASSAY14	Rat sample 02	ID14	Analyte	1.000	2.82	868.267		868.267	bb	1.2	17.9
15	ASSAY15	Rat sample 03	ID15	Analyte	1.000	2.82	571.341		571.341	bb	0.8	-22.4
16	ASSAY16	Rat sample 04	ID16	Analyte	1.000	2.82	817.599		817.599	bb	1.1	11.0
17	ASSAY17	Rat sample 05	ID17	Analyte	1.000	2.82	817.181		817.181	bb	1.1	10.9
18	ASSAY18	Rat sample 06	ID18	Analyte	1.000	2.82	727.503		727.503	bb	1.0	-1.2
19	ASSAY19	Rat sample 07	ID19	Analyte	1.000	2.82	924.266		924.266	bb	1.3	25.5
20	ASSAY20	Rat sample 08	ID20	Analyte	1.000	2.82	641.667		641.667	bb	0.9	-12.9
21	ASSAY21	Rat sample 09	ID21	Analyte	1.000	2.82	690.912		690.912	bb	0.9	-6.2
22	ASSAY22	Rat sample 10	ID22	Analyte	1.000	2.82	692.057		692.057	bb	0.9	-6.0
23	ASSAY23	Rat sample 11	ID23	Analyte	1.000	2.82	890.020		890.020	bb	1.2	20.8
24	ASSAY24	Rat sample 12	ID24	Analyte	1.000	2.82	835.173		835.173	bb	1.1	13.4
25	ASSAY25	Rat sample 13	ID25	Analyte	1.000	2.82	372.693		372.693	bb	0.5	-49.4
26	ASSAY26	Rat sample 14	ID26	Analyte	1.000	2.82	816.763		816.763	bb	1.1	10.9
27	ASSAY27	Rat sample 15	ID27	Analyte	1.000	2.82	733.391		733.391	bb	1.0	-0.4
28	ASSAY28	Ratsample 16	ID28	Analyte	1.000	2.82	736.818		736.818	bb	1.0	0.0
29	ASSAY29	Rat sample 17	ID29	Analyte	1.000	2.84	782.950		782.950	bb	1.1	6.3
30	ASSAY30	12pg/ml QC	ID60	QC	1.000	2.81	1263.891		1263.891	bb	1.7	71.6
31	ASSAY31	plasma blank	ID61	Blank	1.000	2.82	13.485		13.485	bb	0.0	-98.2
32	ASSAY32	0.2pg/ml std	ID62	Standard	1.000	2.81	685.769		685.769	bb	0.9	-6.9
33	ASSAY33	0.5pg/ml std	ID63	Standard	1.000	2.81	683.362		683.362	bd	0.9	-7.2
34	ASSAY34	0.75pg/ml std	ID64	Standard	1.000	2.81	699.598		699.598	bb	0.9	-5.0
35	ASSAY35	1pg/ml std	ID65	Standard	1.000	2.81	417.738		417.738	bb	0.6	-43.3
36	ASSAY36	2pg/ml std	ID66	Standard	1.000	2.81	761.854		761.854	bd	1.0	3.4
37	ASSAY37	5pg/ml std	ID67	Standard	1.000	2.81	740.750		740.750	bb	1.0	0.6
38	ASSAY38	10pg/ml std	ID68	Standard	1.000	2.79	480.845		480.845	bb	0.7	-34.7
39	ASSAY39	15pg/ml std	ID69	Standard	1.000	2.81	745.369		745.369	bb	1.0	1.2

Table 2 1 Std results summary

Checked _____ Date _____

Now click on the **Next Compound** button  on the QuanLynx toolbar so that the Results Summary for the **Parent** are displayed. Check that the data matches that shown in Table 3 below.

#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
1	ASSAY01	plasma blank	ID	Blank				930.147				
2	ASSAY02	0.2pg/ml std	ID2	Standard	0.200	2.79	101.248	883.674	0.115	bb	0.2	6.3
3	ASSAY03	0.5pg/ml std	ID3	Standard	0.500	2.79	230.660	808.750	0.285	bb	0.5	3.9
4	ASSAY04	0.75pg/ml std	ID4	Standard	0.750	2.79	294.603	753.757	0.391	bb	0.7	-5.3
5	ASSAY05	1pg/ml std	ID5	Standard	1.000	2.79	415.267	759.225	0.547	bb	1.0	-0.9
6	ASSAY06	2pg/ml std	ID6	Standard	2.000	2.79	869.522	824.580	1.055	bb	1.9	-4.8
7	ASSAY07	5pg/ml std	ID7	Standard	5.000	2.79	2486.259	916.398	2.713	bb	4.9	-2.2
8	ASSAY08	10pg/ml std	ID8	Standard	10.000	2.81	4816.926	822.216	5.858	bb	10.6	5.5
9	ASSAY09	15pg/ml std	ID9	Standard	15.000	2.79	6389.434	801.626	7.971	bb	14.4	-4.3
10	ASSAY10	0.3pg/ml QC	ID10	QC	0.300	2.79	142.058	776.791	0.183	bb	0.3	11.8
11	ASSAY11	2pg/ml QC	ID11	QC	2.000	2.79	1089.498	1063.683	1.024	bd	1.9	-7.5
12	ASSAY12	12pg/ml QC	ID12	QC	12.000	2.81	4869.008	804.075	6.055	bb	10.9	-9.1
13	ASSAY13	Rat sample 01	ID13	Blank		2.82	7.917	972.523	0.008	bb	0.0	
14	ASSAY14	Rat sample 02	ID14	Analyte		2.82	1592.845	868.267	1.835	bb	3.3	
15	ASSAY15	Rat sample 03	ID15	Analyte		2.82	1166.141	571.341	2.041	bb	3.7	
16	ASSAY16	Rat sample 04	ID16	Analyte		2.82	1706.613	817.599	2.087	bb	3.8	
17	ASSAY17	Rat sample 05	ID17	Analyte		2.82	890.391	817.181	1.090	bb	2.0	
18	ASSAY18	Rat sample 06	ID18	Analyte		2.82	800.093	727.503	1.100	bb	2.0	
19	ASSAY19	Rat sample 07	ID19	Analyte		2.82	518.311	924.266	0.561	bb	1.0	
20	ASSAY20	Rat sample 08	ID20	Analyte		2.82	1461.135	641.667	2.277	bb	4.1	
21	ASSAY21	Rat sample 09	ID21	Analyte		2.82	2032.159	690.912	2.941	bb	5.3	
22	ASSAY22	Rat sample 10	ID22	Analyte		2.82	224.831	692.057	0.325	bb	0.6	
23	ASSAY23	Rat sample 11	ID23	Analyte		2.82	1986.594	890.020	2.232	bb	4.0	
24	ASSAY24	Rat sample 12	ID24	Analyte		2.82	1078.103	835.173	1.291	bb	2.3	
25	ASSAY25	Rat sample 13	ID25	Analyte		2.82	682.509	372.693	1.831	bb	3.3	
26	ASSAY26	Rat sample 14	ID26	Analyte		2.82	2963.864	816.763	3.629	bb	6.5	
27	ASSAY27	Rat sample 15	ID27	Analyte		2.82	1715.681	733.391	2.339	bb	4.2	
28	ASSAY28	Ratsample 16	ID28	Analyte		2.82	1774.026	736.818	2.408	bb	4.3	
29	ASSAY29	Rat sample 17	ID29	Analyte		2.84	2982.158	782.950	3.809	bb	6.9	
30	ASSAY30	12pg/ml QC	ID60	QC	12.000	2.81	7269.290	1263.891	5.752	bb	10.4	-13.7
31	ASSAY31	plasma blank	ID61	Blank				13.485				
32	ASSAY32	0.2pg/ml std	ID62	Standard	0.200	2.81	76.763	685.769	0.112	bb	0.2	3.9
33	ASSAY33	0.5pg/ml std	ID63	Standard	0.500	2.81	180.399	683.362	0.264	bb	0.5	-3.7
34	ASSAY34	0.75pg/ml std	ID64	Standard	0.750	2.81	281.372	699.598	0.402	bb	0.7	-2.6
35	ASSAY35	1pg/ml std	ID65	Standard	1.000	2.81	227.031	417.738	0.543	bb	1.0	-1.5
36	ASSAY36	2pg/ml std	ID66	Standard	2.000	2.81	850.115	761.854	1.116	bd	2.0	0.8
37	ASSAY37	5pg/ml std	ID67	Standard	5.000	2.81	2059.490	740.750	2.780	bb	5.0	0.2
38	ASSAY38	10pg/ml std	ID68	Standard	10.000	2.79	2870.795	480.845	5.970	bb	10.8	7.5
39	ASSAY39	15pg/ml std	ID69	Standard	15.000	2.81	6032.892	745.369	8.094	bb	14.6	-2.8

Table 3 Parent results summary

Checked _____ Date _____

Finally click the **Next Compound** button  again to display the **Metabolite** Results Summary. Check that the data matches that shown in Table 4 below.

#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
1	ASSAY01	plasma blank	ID	Blank				930.147				
2	ASSAY02	0.2pg/ml std	ID2	Standard	0.200	2.63	55.113	883.674	0.062	bb	0.2	11.2
3	ASSAY03	0.5pg/ml std	ID3	Standard	0.500	2.65	132.166	808.750	0.163	bb	0.6	14.4
4	ASSAY04	0.75pg/ml std	ID4	Standard	0.750	2.65	146.236	753.757	0.194	bb	0.7	-9.6
5	ASSAY05	1pg/ml std	ID5	Standard	1.000	2.65	204.967	759.225	0.270	bb	0.9	-5.9
6	ASSAY06	2pg/ml std	ID6	Standard	2.000	2.65	479.941	824.580	0.582	bb	2.0	1.1
7	ASSAY07	5pg/ml std	ID7	Standard	5.000	2.65	1302.141	916.398	1.421	bb	4.9	-1.5
8	ASSAY08	10pg/ml std	ID8	Standard	10.000	2.65	2556.472	822.216	3.109	bb	10.8	7.7
9	ASSAY09	15pg/ml std	ID9	Standard	15.000	2.65	3423.765	801.626	4.271	bb	14.8	-1.4
10	ASSAY10	0.3pg/ml QC	ID10	QC	0.300	2.63	62.630	776.791	0.081	bb	0.3	-4.8
11	ASSAY11	2pg/ml QC	ID11	QC	2.000	2.65	552.174	1063.683	0.519	bb	1.8	-9.8
12	ASSAY12	12pg/ml QC	ID12	QC	12.000	2.65	2507.856	804.075	3.119	bb	10.8	-10
13	ASSAY13	Rat sample 01	ID13	Blank				972.523				
14	ASSAY14	Rat sample 02	ID14	Analyte		2.66	804.966	868.267	0.927	bb	3.2	
15	ASSAY15	Rat sample 03	ID15	Analyte		2.66	657.440	571.341	1.151	bb	4.0	
16	ASSAY16	Rat sample 04	ID16	Analyte		2.66	962.961	817.599	1.178	bb	4.1	
17	ASSAY17	Rat sample 05	ID17	Analyte		2.66	515.624	817.181	0.631	bb	2.2	
18	ASSAY18	Rat sample 06	ID18	Analyte		2.66	444.401	727.503	0.611	bb	2.1	
19	ASSAY19	Rat sample 07	ID19	Analyte		2.68	258.598	924.266	0.280	bb	1.0	
20	ASSAY20	Rat sample 08	ID20	Analyte		2.66	813.804	641.667	1.268	bb	4.4	
21	ASSAY21	Rat sample 09	ID21	Analyte		2.68	1184.878	690.912	1.715	bb	5.9	
22	ASSAY22	Rat sample 10	ID22	Analyte		2.68	113.328	692.057	0.164	bb	0.6	
23	ASSAY23	Rat sample 11	ID23	Analyte		2.68	1195.566	890.020	1.343	bb	4.7	
24	ASSAY24	Rat sample 12	ID24	Analyte		2.68	617.358	835.173	0.739	bb	2.6	
25	ASSAY25	Rat sample 13	ID25	Analyte		2.68	363.418	372.693	0.975	bb	3.4	
26	ASSAY26	Rat sample 14	ID26	Analyte		2.68	1683.629	816.763	2.061	bb	7.1	
27	ASSAY27	Rat sample 15	ID27	Analyte		2.68	1046.488	733.391	1.427	bb	4.9	
28	ASSAY28	Ratsample 16	ID28	Analyte		2.68	1040.561	736.818	1.412	bb	4.9	
29	ASSAY29	Rat sample 17	ID29	Analyte		2.68	1578.182	782.950	2.016	bb	7.0	
30	ASSAY30	12pg/ml QC	ID60	QC	12.000	2.66	2806.523	1263.891	2.221	bb	7.7	-35.9
31	ASSAY31	plasma blank	ID61	Blank				13.485				
32	ASSAY32	0.2pg/ml std	ID62	Standard	0.200	2.66	38.468	685.769	0.056	bb	0.2	0.4
33	ASSAY33	0.5pg/ml std	ID63	Standard	0.500	2.66	103.563	683.362	0.152	bb	0.5	6.2
34	ASSAY34	0.75pg/ml std	ID64	Standard	0.750	2.66	136.094	699.598	0.195	bb	0.7	-9.3
35	ASSAY35	1pg/ml std	ID65	Standard	1.000	2.65	115.758	417.738	0.277	bb	1.0	-3.4
36	ASSAY36	2pg/ml std	ID66	Standard	2.000	2.66	401.251	761.854	0.527	bb	1.8	-8.5
37	ASSAY37	5pg/ml std	ID67	Standard	5.000	2.66	1039.202	740.750	1.403	bb	4.9	-2.8
38	ASSAY38	10pg/ml std	ID68	Standard	10.000	2.65	1472.140	480.845	3.062	bb	10.6	6.0
39	ASSAY39	15pg/ml std	ID69	Standard	15.000	2.66	3080.854	745.369	4.133	bb	14.3	-4.6

Table 4 Metabolite results summary

Checked _____ Date _____

9.6 Editing Calibration Curves

With the **1. Std** compound selected in the QuanLynx Browser click on the **Toggle Chromatogram** button  to remove the Chromatogram window. Then maximise the Calibration window so that the Browser appears as in Figure 4 shown below.

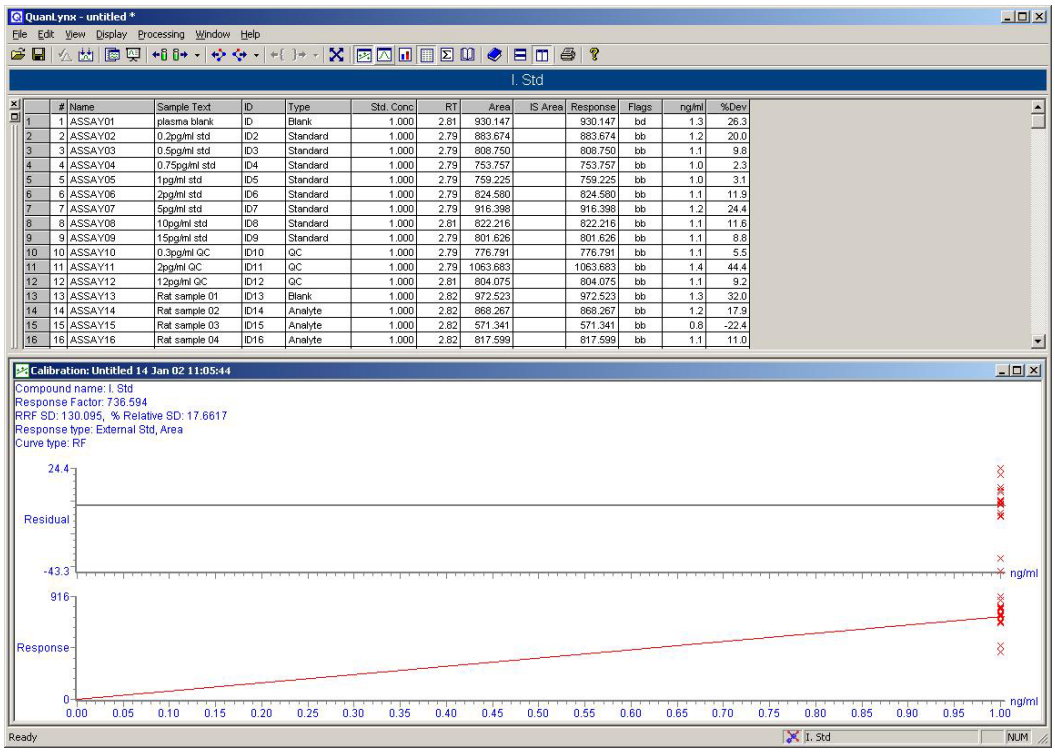


Figure 4 QuanLynx calibration

Note: The Calibration Window title will not be identical to this example.

Checked _____ Date _____

Click on the **Next Compound** button  until the **Metabolite** compound is displayed. Right click on the Calibration Graph and select **Display Options…** Set the display settings to those shown in Figure 5 below. Then click **OK**.

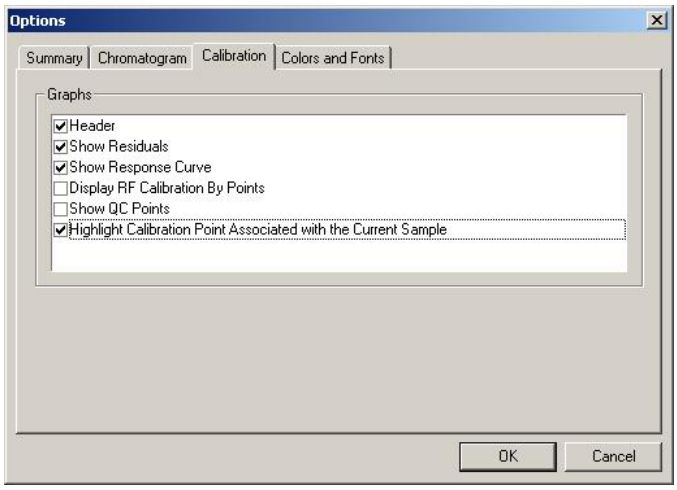


Figure 5 Calibration display settings

Checked _____ Date _____

Now in the Results Summary right click on ASSAY08 and select **Show Chromatograms**. The corresponding calibration point will now be highlighted on the Calibration Graph. Right click on the point and select **Exclude**. A dialog asking if you wish to add an Alteration Comment will appear as shown in Figure 6 below. Click **No** to dismiss this dialog.

Figure 6 Alteration comment

Note the appearance of this dialog (Figure 6) will depend upon whether security is installed with MassLynx and if so what level of security is enabled. In these instances take appropriate actions to accept the changes.
If MassLynx Security is installed, depending on the security level, it is not be possible to modify the results until the Dataset has been saved to disk. In this case use the File Save command to save the Dataset, before performing the exclude operation. The QuanLynx window title will then reflect the name of the saved file.

Checked _____ Date _____

Repeat this for ASSAY38 so that the QuanLynx Browser appears as shown in Figure 7 below.

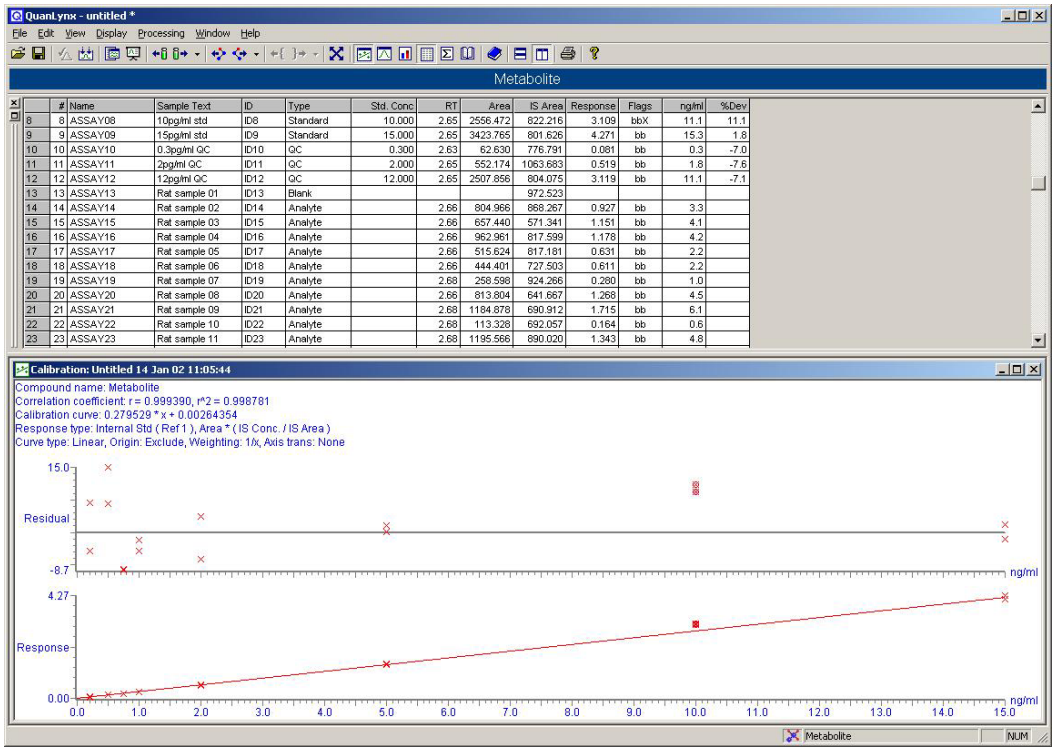
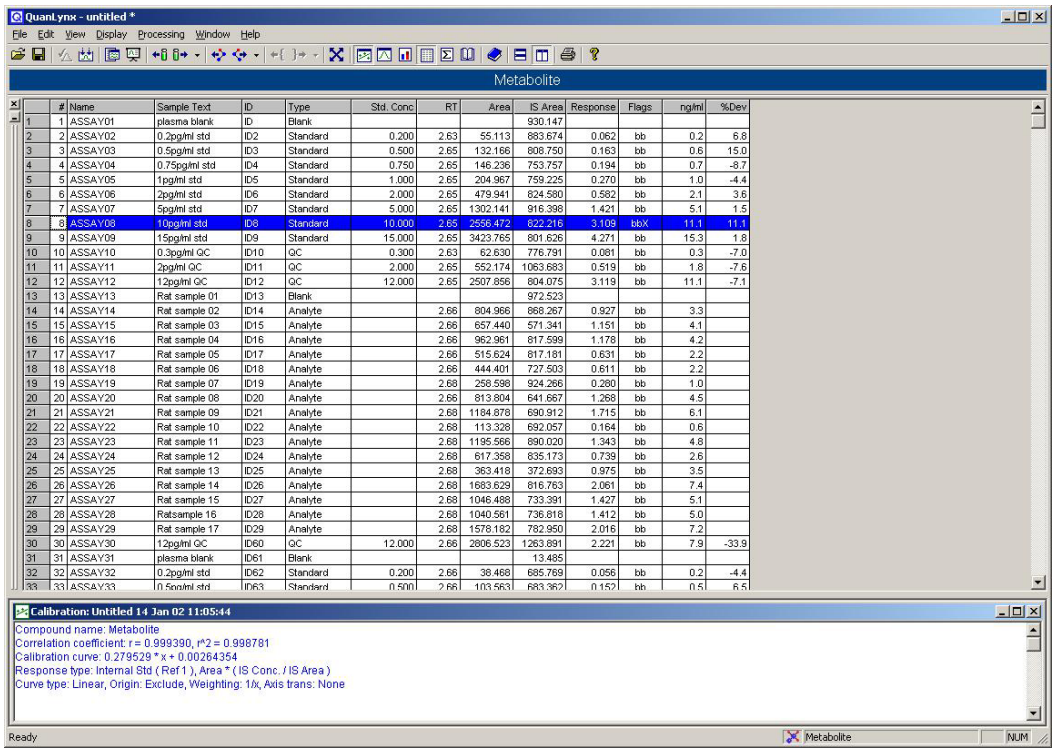


Figure 7 ASSAY08 and ASSAY38 excluded

Checked _____ Date _____

Now click on the Maximise button for the Results Summary located in the top left corner of the window. The display should now appear as shown in Figure 8 below.



	#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
1	1	ASSAY01	plasma blank	ID	Blank				930.147				
2	2	ASSAY02	0.2pg/ml std	ID2	Standard	0.200	2.63	55.113	883.674	0.062	bb	0.2	6.8
3	3	ASSAY03	0.5pg/ml std	ID3	Standard	0.500	2.65	132.166	808.750	0.163	bb	0.6	15.0
4	4	ASSAY04	0.75pg/ml std	ID4	Standard	0.750	2.65	146.236	753.757	0.194	bb	0.7	-8.7
5	5	ASSAY05	1pg/ml std	ID5	Standard	1.000	2.65	204.967	759.225	0.270	bb	1.0	-4.4
6	6	ASSAY06	2pg/ml std	ID6	Standard	2.000	2.65	479.941	824.580	0.582	bb	2.1	3.6
7	7	ASSAY07	5pg/ml std	ID7	Standard	5.000	2.65	1302.141	916.398	1.421	bb	5.1	1.5
8	8	ASSAY08	10pg/ml std	ID8	Standard	10.000	2.65	2556.472	822.216	3.109	bbX	11.1	11.1
9	9	ASSAY09	15pg/ml std	ID9	Standard	15.000	2.65	3423.765	801.626	4.271	bb	15.3	1.8
10	10	ASSAY10	0.3pg/ml QC	ID10	QC	0.300	2.63	62.630	776.791	0.081	bb	0.3	-7.0
11	11	ASSAY11	2pg/ml QC	ID11	QC	2.000	2.65	552.174	1063.683	0.519	bb	1.8	-7.6
12	12	ASSAY12	12pg/ml QC	ID12	QC	12.000	2.65	2507.856	804.075	3.119	bb	11.1	-7.1
13	13	ASSAY13	Rat sample 01	ID13	Blank				972.523				
14	14	ASSAY14	Rat sample 02	ID14	Analyte		2.66	804.966	868.267	0.927	bb	3.3	
15	15	ASSAY15	Rat sample 03	ID15	Analyte		2.66	657.440	571.341	1.151	bb	4.1	
16	16	ASSAY16	Rat sample 04	ID16	Analyte		2.66	962.961	817.599	1.178	bb	4.2	
17	17	ASSAY17	Rat sample 05	ID17	Analyte		2.66	515.624	817.181	0.631	bb	2.2	
18	18	ASSAY18	Rat sample 06	ID18	Analyte		2.66	444.401	727.503	0.611	bb	2.2	
19	19	ASSAY19	Rat sample 07	ID19	Analyte		2.68	258.598	924.266	0.280	bb	1.0	
20	20	ASSAY20	Rat sample 08	ID20	Analyte		2.66	813.804	641.667	1.268	bb	4.5	
21	21	ASSAY21	Rat sample 09	ID21	Analyte		2.68	1184.878	690.912	1.715	bb	6.1	
22	22	ASSAY22	Rat sample 10	ID22	Analyte		2.68	113.328	692.057	0.164	bb	0.6	
23	23	ASSAY23	Rat sample 11	ID23	Analyte		2.68	1195.566	890.020	1.343	bb	4.8	
24	24	ASSAY24	Rat sample 12	ID24	Analyte		2.68	617.358	835.173	0.739	bb	2.6	
25	25	ASSAY25	Rat sample 13	ID25	Analyte		2.68	363.418	372.693	0.975	bb	3.5	
26	26	ASSAY26	Rat sample 14	ID26	Analyte		2.68	1683.629	816.763	2.061	bb	7.4	
27	27	ASSAY27	Rat sample 15	ID27	Analyte		2.68	1046.488	733.391	1.427	bb	5.1	
28	28	ASSAY28	Ratsample 16	ID28	Analyte		2.68	1040.561	736.818	1.412	bb	5.0	
29	29	ASSAY29	Rat sample 17	ID29	Analyte		2.68	1578.182	782.950	2.016	bb	7.2	
30	30	ASSAY30	12pg/ml QC	ID60	QC	12.000	2.66	2806.523	1263.891	2.221	bb	7.9	-33.9
31	31	ASSAY31	plasma blank	ID61	Blank				13.485				
32	32	ASSAY32	0.2pg/ml std	ID62	Standard	0.200	2.66	38.468	685.769	0.056	bb	0.2	-4.4
33	33	ASSAY33	0.5pg/ml std	ID63	Standard	0.500	2.66	103.563	683.362	0.152	bb	0.5	6.5

Figure 8 Results summary maximised

Checked _____ Date _____

Check that the data matches that shown in Table 5 below.

#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
1	ASSAY01	plasma blank	ID	Blank				930.147				
2	ASSAY02	0.2pg/ml std	ID2	Standard	0.200	2.63	55.113	883.674	0.062	bb	0.2	6.8
3	ASSAY03	0.5pg/ml std	ID3	Standard	0.500	2.65	132.166	808.750	0.163	bb	0.6	15
4	ASSAY04	0.75pg/ml std	ID4	Standard	0.750	2.65	146.236	753.757	0.194	bb	0.7	-8.7
5	ASSAY05	1pg/ml std	ID5	Standard	1.000	2.65	204.967	759.225	0.270	bb	1.0	-4.4
6	ASSAY06	2pg/ml std	ID6	Standard	2.000	2.65	479.941	824.580	0.582	bb	2.1	3.6
7	ASSAY07	5pg/ml std	ID7	Standard	5.000	2.65	1302.141	916.398	1.421	bb	5.1	1.5
8	ASSAY08	10pg/ml std	ID8	Standard	10.000	2.65	2556.472	822.216	3.109	bbX	11.1	11.1
9	ASSAY09	15pg/ml std	ID9	Standard	15.000	2.65	3423.765	801.626	4.271	bb	15.3	1.8
10	ASSAY10	0.3pg/ml QC	ID10	QC	0.300	2.63	62.630	776.791	0.081	bb	0.3	-7.0
11	ASSAY11	2pg/ml QC	ID11	QC	2.000	2.65	552.174	1063.683	0.519	bb	1.8	-7.6
12	ASSAY12	12pg/ml QC	ID12	QC	12.000	2.65	2507.856	804.075	3.119	bb	11.1	-7.1
13	ASSAY13	Rat sample 01	ID13	Blank				972.523				
14	ASSAY14	Rat sample 02	ID14	Analyte		2.66	804.966	868.267	0.927	bb	3.3	
15	ASSAY15	Rat sample 03	ID15	Analyte		2.66	657.440	571.341	1.151	bb	4.1	
16	ASSAY16	Rat sample 04	ID16	Analyte		2.66	962.961	817.599	1.178	bb	4.2	
17	ASSAY17	Rat sample 05	ID17	Analyte		2.66	515.624	817.181	0.631	bb	2.2	
18	ASSAY18	Rat sample 06	ID18	Analyte		2.66	444.401	727.503	0.611	bb	2.2	
19	ASSAY19	Rat sample 07	ID19	Analyte		2.68	258.598	924.266	0.280	bb	1.0	
20	ASSAY20	Rat sample 08	ID20	Analyte		2.66	813.804	641.667	1.268	bb	4.5	
21	ASSAY21	Rat sample 09	ID21	Analyte		2.68	1184.878	690.912	1.715	bb	6.1	
22	ASSAY22	Rat sample 10	ID22	Analyte		2.68	113.328	692.057	0.164	bb	0.6	
23	ASSAY23	Rat sample 11	ID23	Analyte		2.68	1195.566	890.020	1.343	bb	4.8	
24	ASSAY24	Rat sample 12	ID24	Analyte		2.68	617.358	835.173	0.739	bb	2.6	
25	ASSAY25	Rat sample 13	ID25	Analyte		2.68	363.418	372.693	0.975	bb	3.5	
26	ASSAY26	Rat sample 14	ID26	Analyte		2.68	1683.629	816.763	2.061	bb	7.4	
27	ASSAY27	Rat sample 15	ID27	Analyte		2.68	1046.488	733.391	1.427	bb	5.1	
28	ASSAY28	Ratsample 16	ID28	Analyte		2.68	1040.561	736.818	1.412	bb	5.0	
29	ASSAY29	Rat sample 17	ID29	Analyte		2.68	1578.182	782.950	2.016	bb	7.2	
30	ASSAY30	12pg/ml QC	ID60	QC	12.000	2.66	2806.523	1263.891	2.221	bb	7.9	-33.9
31	ASSAY31	plasma blank	ID61	Blank				13.485				
32	ASSAY32	0.2pg/ml std	ID62	Standard	0.200	2.66	38.468	685.769	0.056	bb	0.2	-4.4
33	ASSAY33	0.5pg/ml std	ID63	Standard	0.500	2.66	103.563	683.362	0.152	bb	0.5	6.5
34	ASSAY34	0.75pg/ml std	ID64	Standard	0.750	2.66	136.094	699.598	0.195	bb	0.7	-8.5
35	ASSAY35	1pg/ml std	ID65	Standard	1.000	2.65	115.758	417.738	0.277	bb	1.0	-1.8
36	ASSAY36	2pg/ml std	ID66	Standard	2.000	2.66	401.251	761.854	0.527	bb	1.9	-6.3
37	ASSAY37	5pg/ml std	ID67	Standard	5.000	2.66	1039.202	740.750	1.403	bb	5.0	0.2
38	ASSAY38	10pg/ml std	ID68	Standard	10.000	2.65	1472.140	480.845	3.062	bbX	10.9	9.4
39	ASSAY39	15pg/ml std	ID69	Standard	15.000	2.66	3080.854	745.369	4.133	bb	14.8	-1.5

Table 5 Altered Metabolite results summary

Checked _____ Date _____

From the QuanLynx Browser menu **File** select **Print…** and set the parameters as shown in Figure 9 below.

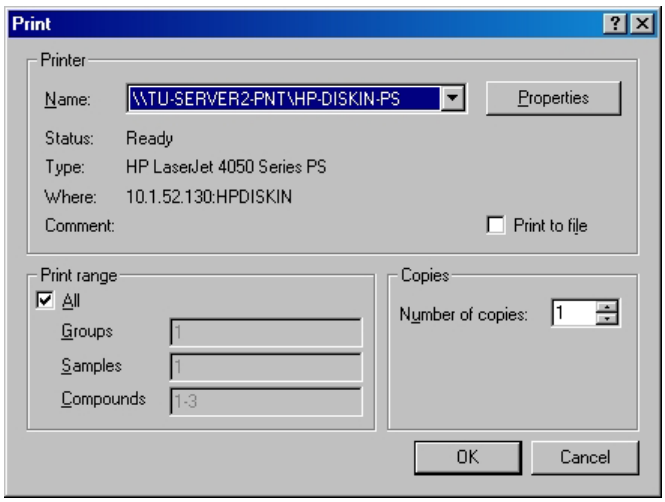


Figure 9 Print

Check that the printed **Metabolite** data in the Compound Summary Report matches that shown in Table 5 previously.

Checked _____ Date _____

9.7 Editing Chromatographic Peaks

In the QuanLynx Browser click on the **Toggle Calibration** button  to remove the Calibration Graph.

Then click on the **Toggle Chromatogram** button  to add the Chromatogram View. Then maximise the Chromatogram View. In the Results Summary right click on ASSAY14 and select **Show Chromatograms**. The Browser should now appear as shown in Figure 10 below.

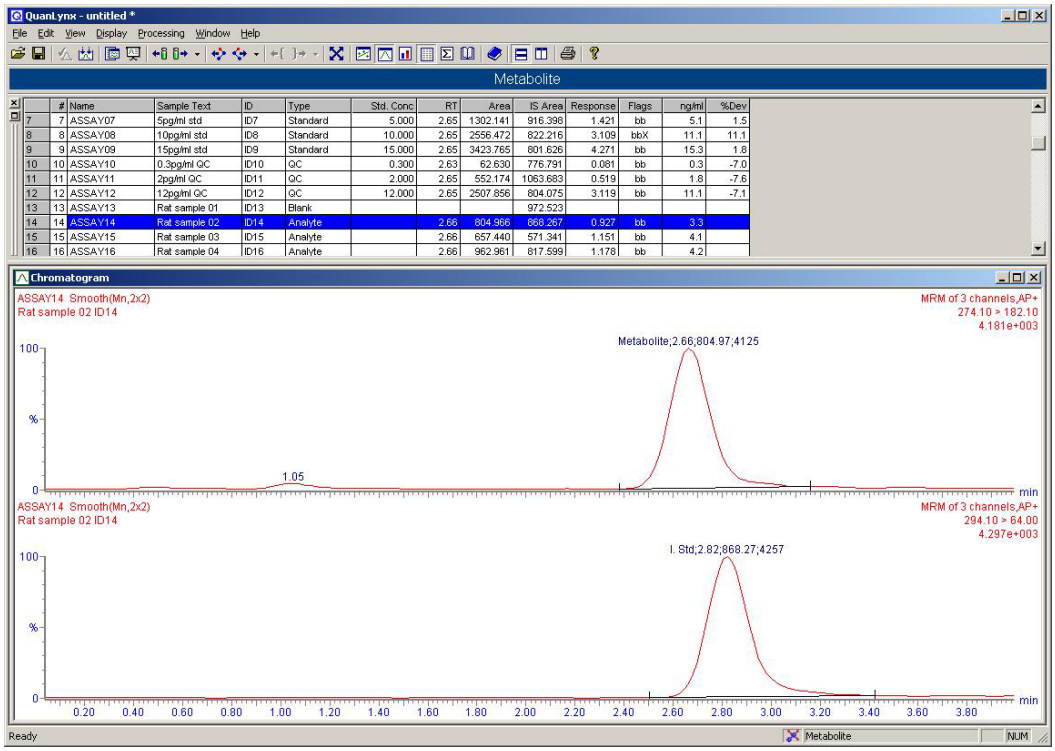
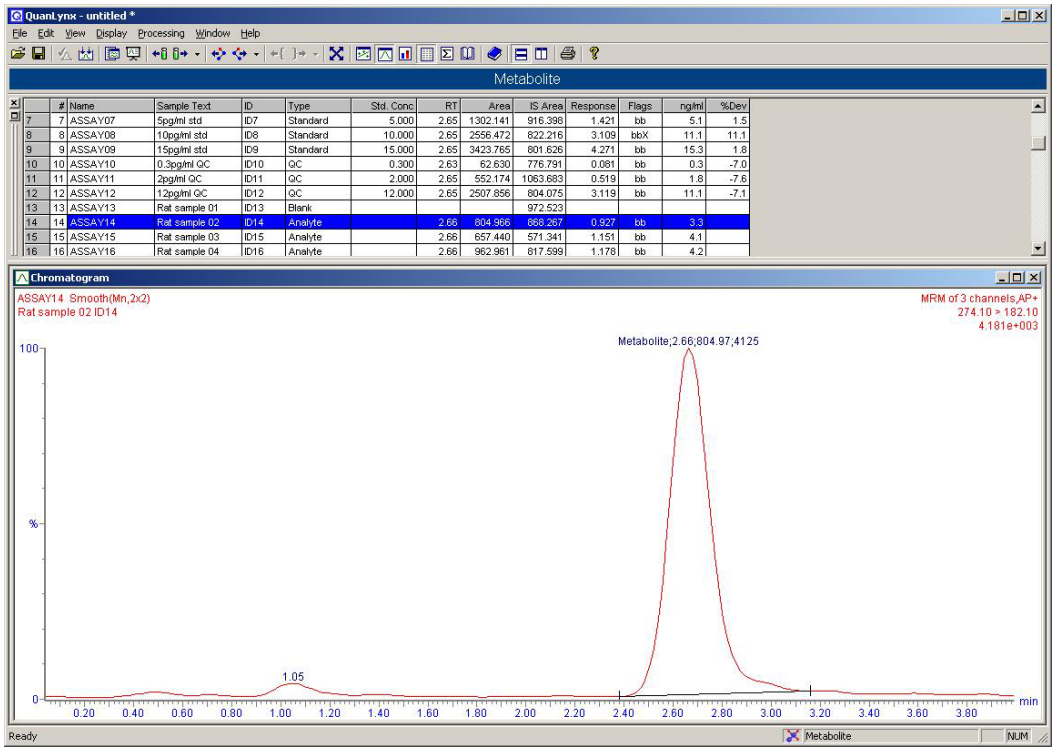


Figure 10 Chromatogram display

Checked _____ Date _____

Right click on the Chromatogram and select **Display Options…** and uncheck the **Show Internal Standard** in the **Style** list. The Browser should now appear as shown in Figure 11 below.

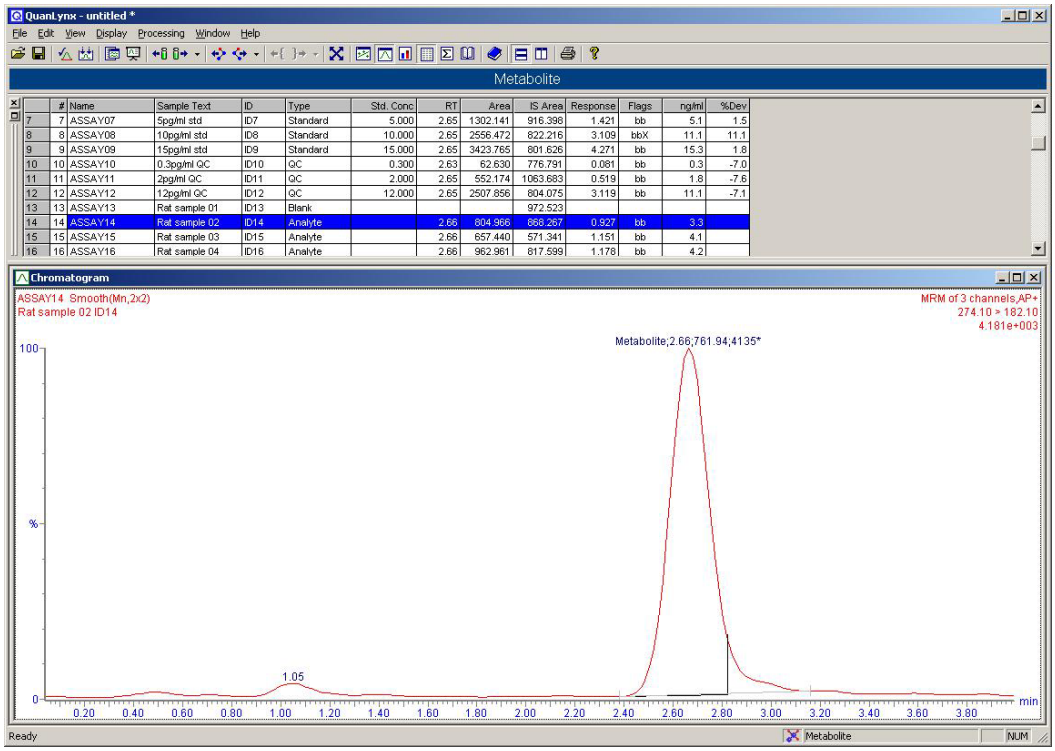


	#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
7	7	ASSAY07	5pg/ml std	ID7	Standard	5.000	2.65	1302.141	916.398	1.421	bb	5.1	1.5
8	8	ASSAY08	10pg/ml std	ID8	Standard	10.000	2.65	2556.472	822.216	3.109	bbX	11.1	11.1
9	9	ASSAY09	15pg/ml std	ID9	Standard	15.000	2.65	3423.765	801.626	4.271	bb	15.3	1.8
10	10	ASSAY10	0.3pg/ml QC	ID10	QC	0.300	2.63	62.630	776.791	0.081	bb	0.3	-7.0
11	11	ASSAY11	2pg/ml QC	ID11	QC	2.000	2.65	552.174	1063.683	0.519	bb	1.8	-7.6
12	12	ASSAY12	12pg/ml QC	ID12	QC	12.000	2.65	2507.856	804.075	3.119	bb	11.1	-7.1
13	13	ASSAY13	Rat sample 01	ID13	Blank				972.523				
14	14	ASSAY14	Rat sample 02	ID14	Analyte		2.66	804.968	868.267	0.927	bb	3.3	
15	15	ASSAY15	Rat sample 03	ID15	Analyte		2.66	657.440	571.341	1.151	bb	4.1	
16	16	ASSAY16	Rat sample 04	ID16	Analyte		2.66	962.961	817.599	1.178	bb	4.2	

Figure 11 Chromatogram with no internal standard

Checked _____ Date _____

Zoom into the peak at 2.66 minutes by left clicking at 2.00 minutes and dragging the cursor to 3.40 minutes. Now left click on the end of the peak baseline at 3.16 minutes and move this back to about 2.81 minutes by dragging the cursor. The Browser should now appear as shown in Figure 12 below. Note that area and height annotation of the peak may vary from that shown.



#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
7	7	ASSAY07	5pg/ml std	ID7	Standard	5.000	2.65	1302.141	916.398	1.421	bb	5.1	1.5
8	8	ASSAY08	10pg/ml std	ID8	Standard	10.000	2.65	2556.472	822.216	3.109	bbX	11.1	11.1
9	9	ASSAY09	15pg/ml std	ID9	Standard	15.000	2.65	3423.765	801.626	4.271	bb	15.3	1.8
10	10	ASSAY10	0.3pg/ml QC	ID10	QC	0.300	2.63	62.630	776.791	0.081	bb	0.3	-7.0
11	11	ASSAY11	2pg/ml QC	ID11	QC	2.000	2.65	552.174	1063.683	0.519	bb	1.8	-7.6
12	12	ASSAY12	12pg/ml QC	ID12	QC	12.000	2.65	2507.856	804.075	3.119	bb	11.1	-7.1
13	13	ASSAY13	Rat sample 01	ID13	Blank				972.523				
14	14	ASSAY14	Rat sample 02	ID14	Analyte		2.66	804.966	868.267	0.927	bb	3.3	
15	15	ASSAY15	Rat sample 03	ID15	Analyte		2.66	657.440	571.341	1.151	bb	4.1	
16	16	ASSAY16	Rat sample 04	ID16	Analyte		2.66	962.961	817.599	1.178	bb	4.2	

Figure 12 Altered peak baseline

Checked _____ Date _____

Right click on the Chromatogram View and select **Save Peak Modifications…** A dialog asking if you wish to add an Alteration Comment will appear as shown in Figure 6. Type in the comment **Modify test** and click **Yes**.
Right click on the Results Summary window and select **Change Column Order…** Press the **Ctrl** key and click on **Modify Comments** and **Modify User** in the left hand list and click the **Add** button. Then click **OK**. The Browser should now appear as shown in Figure 13 below.

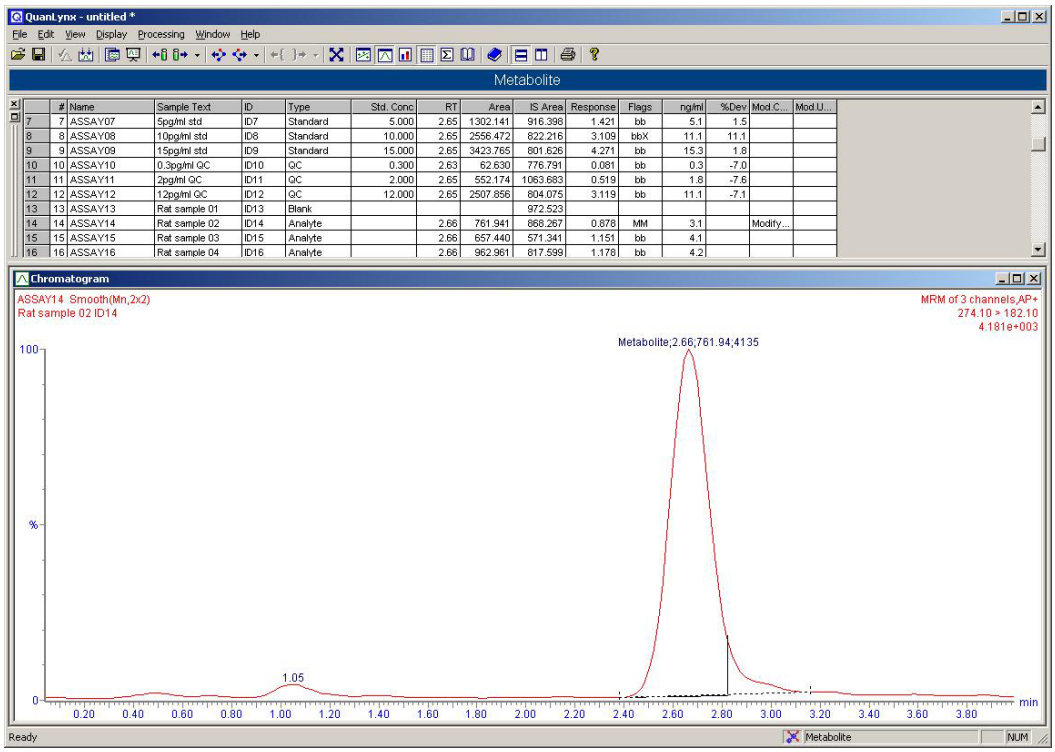


Figure 13 Modified chromatogram

Checked _____ Date _____

NOTE For this Figure there may be slight differences between the one shown above and the actual screen display. These differences will be in the **Mod. User** column, as this will display the name of the user logged into MassLynx, and the **Area, Response** and **ng/m**l columns as these values for ASSAY14 will depend on the actual peak end that has been positioned manually.

9.8 Modifying the Quantification Method

Right click on the Results Summary window and select **Change Column Order…** Press the **Ctrl** key and click on **Modify Comments** and **Modify User**, in the right hand list, then click the **Remove** button. Then click **OK**.
From the QuanLynx menu **Edit** select **Method**. Then in the **Method Editor** dialog click the **General Parameters…** button. Set the parameters as shown in Figure 14 below and click **OK**.

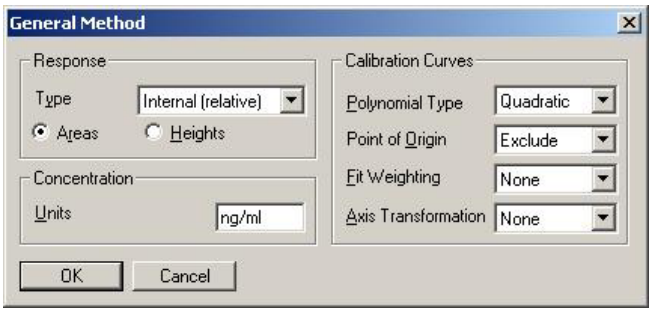


Figure 14 New method parameters

Now close the QuanLynx **Method Editor** and click **Yes** when asked to **Save changes**. A dialog asking if you wish to add an Alteration Comment will appear as shown in Figure 6. Click **No** to dismiss this dialog.

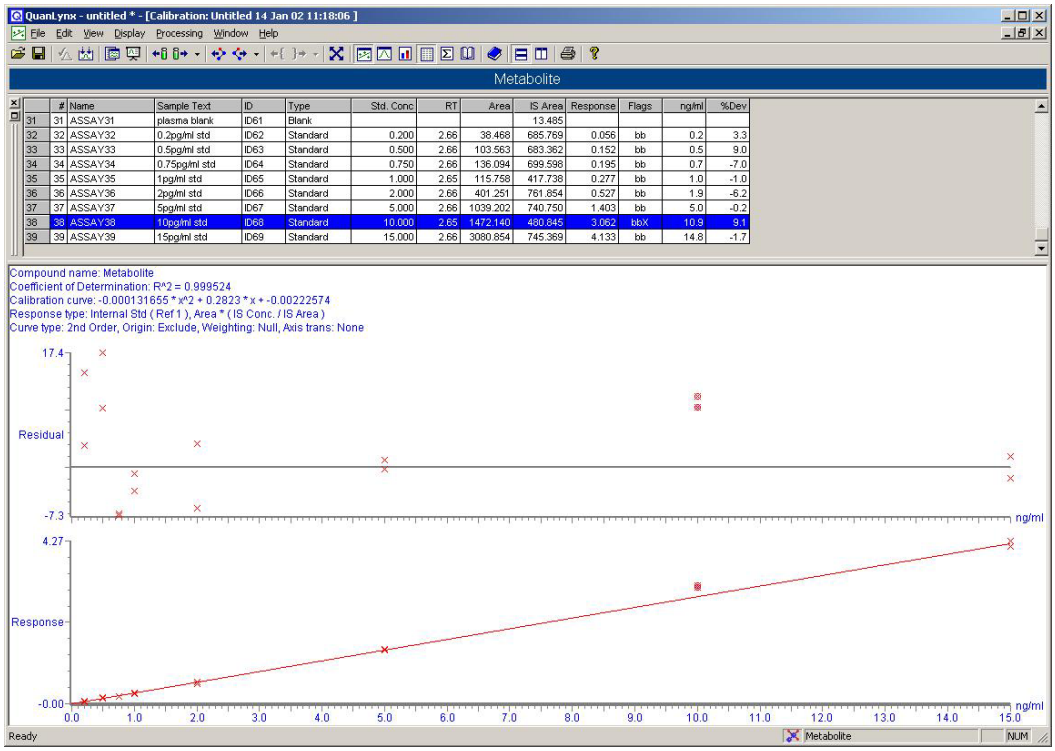
Note the appearance of the Comment dialog will depend upon whether Security is installed. The appropriate action to accept the change should be made.

Checked _____ Date _____

In the QuanLynx Browser click on the **Toggle Chromatogram** button  to remove the Chromatogram View. Then click on the **Toggle Calibration** button  to add the Calibration Graph. Then maximise the Calibration View. From the QuanLynx menu **Processing** select **Execute** and check the options shown in Figure 15 below.

Figure 15 Processing wizard

The Browser should now appear as shown in Figure 16 below.



	#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
31	31	ASSAY31	plasma blank	ID61	Blank				13.485				
32	32	ASSAY32	0.2pg/ml std	ID62	Standard	0.200	2.66	38.468	685.769	0.056	bb	0.2	3.3
33	33	ASSAY33	0.5pg/ml std	ID63	Standard	0.500	2.66	103.563	683.362	0.152	bb	0.5	9.0
34	34	ASSAY34	0.75pg/ml std	ID64	Standard	0.750	2.66	136.094	699.598	0.195	bb	0.7	-7.0
35	35	ASSAY35	1pg/ml std	ID65	Standard	1.000	2.65	115.758	417.738	0.277	bb	1.0	-1.0
36	36	ASSAY36	2pg/ml std	ID66	Standard	2.000	2.66	401.251	761.854	0.527	bb	1.9	-6.2
37	37	ASSAY37	5pg/ml std	ID67	Standard	5.000	2.66	1039.202	740.750	1.403	bb	5.0	-0.2
38	38	ASSAY38	10pg/ml std	ID68	Standard	10.000	2.65	1472.140	480.845	3.062	bbX	10.9	9.1
39	39	ASSAY39	15pg/ml std	ID69	Standard	15.000	2.66	3080.854	745.369	4.133	bb	14.8	-1.7

Figure 16 Quadratic calibration

Checked _____ Date _____

The Results Summary for the **Metabolite** should now match the data shown in Table 6 below. Note that ASSAY14 may display different results due to the previous manual modification.

#	Name	Sample Text	ID	Type	Std. Conc	RT	Area	IS Area	Response	Flags	ng/ml	%Dev
1	ASSAY01	plasma blank	ID	Blank				930.147				
2	ASSAY02	0.2pg/ml std	ID2	Standard	0.200	2.63	55.113	883.674	0.062	bb	0.2	14.4
3	ASSAY03	0.5pg/ml std	ID3	Standard	0.500	2.65	132.166	808.750	0.163	bb	0.6	17.4
4	ASSAY04	0.75pg/ml std	ID4	Standard	0.750	2.65	146.236	753.757	0.194	bb	0.7	-7.3
5	ASSAY05	1pg/ml std	ID5	Standard	1.000	2.65	204.967	759.225	0.270	bb	1.0	-3.5
6	ASSAY06	2pg/ml std	ID6	Standard	2.000	2.65	479.941	824.580	0.582	bb	2.1	3.6
7	ASSAY07	5pg/ml std	ID7	Standard	5.000	2.65	1302.141	916.398	1.421	bb	5.1	1.1
8	ASSAY08	10pg/ml std	ID8	Standard	10.000	2.65	2556.472	822.216	3.109	bbX	11.1	10.8
9	ASSAY09	15pg/ml std	ID9	Standard	15.000	2.65	3423.765	801.626	4.271	bb	15.2	1.6
10	ASSAY10	0.3pg/ml QC	ID10	QC	0.300	2.63	62.630	776.791	0.081	bb	0.3	-2.2
11	ASSAY11	2pg/ml QC	ID11	QC	2.000	2.65	552.174	1063.683	0.519	bb	1.8	-7.6
12	ASSAY12	12pg/ml QC	ID12	QC	12.000	2.65	2507.856	804.075	3.119	bb	11.1	-7.4
13	ASSAY13	Rat sample 01	ID13	Blank				972.523				
14	ASSAY14	Rat sample 02	ID14	Analyte		2.66	804.966	868.267	0.927	mm-	3.3	
15	ASSAY15	Rat sample 03	ID15	Analyte		2.66	657.440	571.341	1.151	bb	4.1	
16	ASSAY16	Rat sample 04	ID16	Analyte		2.66	962.961	817.599	1.178	bb	4.2	
17	ASSAY17	Rat sample 05	ID17	Analyte		2.66	515.624	817.181	0.631	bb	2.2	
18	ASSAY18	Rat sample 06	ID18	Analyte		2.66	444.401	727.503	0.611	bb	2.2	
19	ASSAY19	Rat sample 07	ID19	Analyte		2.68	258.598	924.266	0.280	bb	1.0	
20	ASSAY20	Rat sample 08	ID20	Analyte		2.66	813.804	641.667	1.268	bb	4.5	
21	ASSAY21	Rat sample 09	ID21	Analyte		2.68	1184.878	690.912	1.715	bb	6.1	
22	ASSAY22	Rat sample 10	ID22	Analyte		2.68	113.328	692.057	0.164	bb	0.6	
23	ASSAY23	Rat sample 11	ID23	Analyte		2.68	1195.566	890.020	1.343	bb	4.8	
24	ASSAY24	Rat sample 12	ID24	Analyte		2.68	617.358	835.173	0.739	bb	2.6	
25	ASSAY25	Rat sample 13	ID25	Analyte		2.68	363.418	372.693	0.975	bb	3.5	
26	ASSAY26	Rat sample 14	ID26	Analyte		2.68	1683.629	816.763	2.061	bb	7.3	
27	ASSAY27	Rat sample 15	ID27	Analyte		2.68	1046.488	733.391	1.427	bb	5.1	
28	ASSAY28	Ratsample 16	ID28	Analyte		2.68	1040.561	736.818	1.412	bb	5.0	
29	ASSAY29	Rat sample 17	ID29	Analyte		2.68	1578.182	782.950	2.016	bb	7.2	
30	ASSAY30	12pg/ml QC	ID60	QC	12.000	2.66	2806.523	1263.891	2.221	bb	7.9	-34.1
31	ASSAY31	plasma blank	ID61	Blank				13.485				
32	ASSAY32	0.2pg/ml std	ID62	Standard	0.200	2.66	38.468	685.769	0.056	bb	0.2	3.3
33	ASSAY33	0.5pg/ml std	ID63	Standard	0.500	2.66	103.563	683.362	0.152	bb	0.5	9.0
34	ASSAY34	0.75pg/ml std	ID64	Standard	0.750	2.66	136.094	699.598	0.195	bb	0.7	-7.0
35	ASSAY35	1pg/ml std	ID65	Standard	1.000	2.65	115.758	417.738	0.277	bb	1.0	-1.0
36	ASSAY36	2pg/ml std	ID66	Standard	2.000	2.66	401.251	761.854	0.527	bb	1.9	-6.2
37	ASSAY37	5pg/ml std	ID67	Standard	5.000	2.66	1039.202	740.750	1.403	bb	5.0	-0.2
38	ASSAY38	10pg/ml std	ID68	Standard	10.000	2.65	1472.140	480.845	3.062	bbX	10.9	9.1
39	ASSAY39	15pg/ml std	ID69	Standard	15.000	2.66	3080.854	745.369	4.133	bb	14.8	-1.7

Table 6 Quadratic calibration results summary

Checked _____ Date _____

10. Test execution summary

Observation No.	Notes	Observation or non-conformance	Related observation

Tested By **Name**___________________ **Signature**_________________ **Date** ___________________

Reviewed By **Name**___________________ **Signature**_________________ **Date** ___________________

11. Appendix - Algorithms used by MassLynx peak integration and quantitation

11.1 Peak Response

There are two main methods of calculating peak response value, these are External Standard and Internal Standard. Both are based on the Absolute Response calculated for a peak.

11.1.1 Absolute Response

Absolute Response is based on either peak Area or Height and the selected combination of compound Primary and Secondary peaks. The examples below are shown for peak Area.

Compound Response Primary

This is the default if no Secondary trace is specified.

Abs.Resp. = Primary Area

Compound Response Secondary

Abs.Resp. = Secondary Area

Compound Response Both

Abs.Resp. = Primary Area + Secondary Area

Where:
Primary Area is the area of a peak calculated by peak detection on the primary chromatogram trace.
Secondary Area is the area of a peak calculated by peak detection on the secondary chromatogram trace.

11.1.2 External Response

Peak Response = Abs.Resp

Where:
Abs.Resp is the absolute response of a peak as calculated in 11.1.1.

11.1.3 Internal Response

$$\text{Peak Response} = \frac{\text{Abs.Resp} * \text{Amount}_I}{\text{Abs.Resp}_I}$$

Where:
Abs.Resp is the absolute response of a peak as calculated in 11.1.1.
Amount_I is the given amount of the Internal Standard in the sample
Abs.Resp_I is the area of the internal standard peak as calculated in 11.1.1.

11.2 Primary / Secondary Peak Ratio

If primary and Secondary traces are specified the ratio of peaks detected on each trace is calculated as below. Ratio is based on either peak Area or Height, the example below is shown for peak Area.

$$\text{Ratio} = \frac{\text{Primary Peak Area}}{\text{Secondary Peak Area}}$$

Where:
Primary Area is the area of a peak calculated by peak detection on the primary chromatogram trace.
Secondary Area is the area of a peak calculated by peak detection on the secondary chromatogram trace.

11.3 Calibration Curve Calculations

MassLynx can fit several types of calibration curves, which are described below.

11.3.1 Weighted Calibration Curves

Calibration points used when fitting curves can be given a weighted importance,
the larger the weighting the more significant a point is treated when the curve is fitted.

Weighting (w_i) of i^{th} calibration point is calculated using one of the following, all w_i are set to 1 for no weighting.

1) $w_i = y_i^{-1}$
2) $w_i = y_i^{-2}$
3) $w_i = x_i^{-1}$
4) $w_i = x_i^{-2}$

Where:
y_i is Y value (response) of i^{th} calibration point
x_i is X value (concentration) of i^{th} calibration point

11.3.2 Include Origin

If Include Origin is selected as a calibration curve type an extra point with zero concentration and response is used in the regression. The extra point has the same weighting as the lowest calibration standard.

11.3.3 Average RF

The calibration curve formed is linear passing through the origin with a gradient equal to the average response values of the calibration points.

$$\text{Average RF} = \frac{Swy}{Sw}$$

Where:

$$Swy = \sum \frac{y_i * w_i}{x_i}$$

$$Sw = \sum w_i$$

y_i is Y value (response) of i^{th} calibration point

x_i is X value (concentration) of i^{th} calibration point

w_i is weighting of i^{th} calibration point, all set to 1 for no weighting.

11.3.4 Linear

The calibration curve is formed by fitting a line using linear regression to a set of calibration points.

$$\text{Gradient} = Swxy / Swxx$$

$$\text{Intercept} = y_{w,mean} - \text{Gradient} * x_{w,mean}$$

Where:

$$y_{w,mean} = \frac{\sum y_i * w_i}{\sum w_i}$$

$$x_{w,mean} = \frac{\sum x_i * w_i}{\sum w_i}$$

$$Swxy = \sum (x_i - x_{w,mean}) * (y_i - y_{w,mean}) * w_i$$

$$Swxx = \sum (x_i - x_{w,mean})^2 * w_i$$

y_i is Y value (response) of i^{th} calibration point

x_i is X value (concentration) of i^{th} calibration point

w_i is weighting of i^{th} calibration point, all set to 1 for no weighting.

If Force Origin is selected a line with zero intercept is fitted.

$$\text{Gradient} = \frac{\sum x_i * y_i * w_i}{\sum x_i^2 * w_i}$$

11.3.5 Quadratic and Higher Order Curves

MassLynx uses a general Least Squares Fit algorithm to regress a polynomial of any order against the calibration points. The method used is outlined below.

Polynomial regression can be described as the fitting of m "independent" variables (Xj, j = 0 to m-1) to a single "dependent" variable y. i.e.

$$\mathbf{y = Xb + e}$$

where **y** is the *n* x 1 vector containing the n y values (y_i), **X** is the *n* x *m* matrix of x values, (x_i^j), **b** is the *m* x 1 vector of regression coefficients (b_i), and **e** is the *n* x 1 vector of residuals from the fit to each y_i value.

The familiar least squares solution for the regression coefficients is given by:

$$\mathbf{b = (X'X)^{-1}X'y}$$

where $^{-1}$ indicates matrix inverse, and ' indicates matrix transpose.

The above equation can then be solved using Gauss-Jordan elimination.

To implement weighted regression **X** and **y** are first multiplied by a diagonal *n* x *n* matrix **P** (i.e. **X** becomes **PX** and **Y** becomes **PY**), before the above equation is solved.

where each element (pij) of **P** is given by:

$$p_{ij} = w_i^{1/2} \quad \text{for } i = j$$

$$p_{ij} = 0 \quad \text{for } i <> k$$

w_i is weighting of i^{th} calibration point, all set to 1 for no weighting.

11.4 Peak Amount Calculations

11.4.1 User Specified Response Factor

If a user response factor if selected within the quantitation method calibration curves are not used. The following calculation is performed to obtain peak amounts.

$$\text{Amount} = \frac{\text{Peak Response}}{\text{Response Factor}}$$

Where:
Peak Response is the response value calculated for a peak.
Response Factor is user entered response factor for that compound.

11.4.2 Average RF Calibration Curve

Amounts are calculated using an Average RF calibration as follows

$$\text{Amount} = \frac{\text{Peak Response}}{\text{Average RF}}$$

Where:
Peak Response is the response value calculated for a peak.
Average RF is average response factor calculated for a set of calibration points.

11.4.3 Linear Calibration Curve

Amounts are calculated using a linear calibration as follows

$$\text{Amount} = \frac{\text{Peak Response - Intercept}}{\text{Gradient}}$$

Where:
Peak Response is the response value calculated for a peak.
Intercept is the intercept calculated for the linear calibration.
Gradient is the gradient calculated for the linear calibration.

11.4.4 Quadratic and Higher Order Calibration Curves

Amounts are calculated by solving the following equation using the Newton-Raphson Method.

$$\text{Peak Response} = P(\text{Amount})$$

Where:
Peak Response is the response value calculated for a peak.
P( ) is the polynomial function calculated for a set of calibration points.

11.4.5 User Parameters

User parameters can be used to multiply or divide the final quantitation results. These factors are entered per sample in the Sample List. If a factor is not specified of zero it is assumed to be one.

$$\text{Final Amount} = \frac{\text{Amount} * \text{User Factor 1} * \text{User Factor 2} * \text{User Factor 3}}{\text{User Divisor 1}}$$

The User Peak Factor is entered per compound in the Quantify Method.

$$\text{Final Amount} = \text{Amount} * \text{User Peak Factor}$$

Notes:

User Multiplication and Divisor factors are not applied to Standard samples.

In MassLynx 3 onwards Sample List fields can be renamed to user requirements. When converting from a pre MassLynx V3.0 sample list the following field mappings occur.

'Initial Amount' now becomes 'User Divisor 1'
'User Factor' now becomes 'User Factor 1'
'Dilution Factor' now becomes 'User Factor 2'
'Extract Volume' now becomes 'User Factor 3'

11.5 Calibration Curve Statistics

11.5.1 Coefficient of Determination

The coefficient and determination is calculated for a regressed calibration curve. In the case of a linear curve it is equivalent to the square of the correlation coefficient and is reported as such.

For each data point a value of y ($y_{i,pred}$) can be predicted from the calibration curve at the position x_i. For each data point a residual between the actual and predicted y value can be calculated as (y_i - $y_{i,pred}$), and the weighted residual sum of squares (RSS) can be calculated as:

$$RSS = \Sigma (y_i - y_{i,pred})^2 * w_i$$

where w_i is weighting of i^{th} calibration point.

The total variation in the data is reflected in the weighted corrected sum of squares (CSS), calculated * as:

$$CSS = \Sigma (y_i - y_{w,mean})^2 * w_i$$

where $y_{w,mean}$ is the weighted mean value of y

$$y_{w,mean} = \frac{\Sigma y_i * w_i}{\Sigma w_i}$$

The model sum of squares (MSS) is the portion of the total variation accounted for by the regression, i.e.

$$MSS = CSS - RSS$$

The coefficient of determination (R^2) is the proportion of the variation accounted for by regression, and is given by the ration of the model sum of squares to the corrected sum of squares, i.e.

$$R^2 = MSS / CSS$$
$$= (CSS - RSS) / CSS$$

11.5.2 Curve Correlation Coefficient

In the case of linear curves the square of the correlation coefficient (r) is equivalent to the coefficient of determination described above and is reported as such.

11.5.3 RRF mean, SD and %RSD for Average RF curves

The calculation of RRF mean, SD and %RSD are detailed in equations below :

$$Mean_{RRF} = \frac{\sum_{N} RRF_i}{N} = \frac{\sum_{N} \frac{Abs.\mathrm{Re}sponse_i}{Conc_i}}{N}$$

$$SD_{RRF} = \sqrt{\frac{\sum_{N} \left(RRF_i - Mean_{RRF}\right)^2}{N-1}}$$

$$\%RSD_{RRF} = \left(\frac{SD_{RRF}}{Mean_{RRF}}\right) \times 100\%$$

11.6 Totals Compounds

Totals compounds are formed from groups of peaks, these can include Named peaks that have been identified by other method compounds and Unnamed peaks which have not been identified. Compounds are identified as being a part of a Group by a shared group name

This section details calculations performed specifically for Totals compounds.

11.6.1 Peak Response

The responses of all peaks that are part of the Totals group are calculated individually.

11.6.2 External Response

See section 11.1.2.

11.6.3 Internal Response

Calculated Internal Response of a peak is based upon the average response of the Internal Standards of the named compounds in the Group.

$$\text{Peak Response} = \frac{\text{Abs.Resp}}{\text{Ave.Named.Resp}_{IS}}$$

Where:

Abs.Resp	= Absolute response of Unnamed peak as calculated in 11.1.1.
$\text{Ave.Named.Resp}_{IS}$	= Average response of group Named peaks Internal Standards. $= (\Sigma_N (\text{AbsResp}_{ISn} / \text{Amount}_{ISn})) / N$
N	= Number of found Named compounds in group
n	= Named compound index
AbsResp_{ISn}	= Absolute response of the n^{th} Named Internal Standard peak as calculated in 11.1.1.
Amount_{ISn}	= Given amount of the n^{th} Named Internal Standard in the sample

11.6.4 Calibration Curve Calculations

The calibration curve for a Totals compound is formed by averaging the calibrations of the Named Compounds in the Group. The Totals curve is used to calculate the concentration of the Unnamed peaks in the Group.

Each coefficient of the Totals curve is calculated as follows:

$$C_i = (\Sigma_N C_{i,n}) / N$$

Where:
N is the number of Named compounds in the Group.
C_i is the i^{th} coefficient of the Totals curve.
$C_{i,n}$ is the i^{th} coefficient of the n^{th} Named compound in the group.

11.6.5 Peak Amount Calculations

Totals concentration is the sum of the concentrations of the selected peaks that make up the group.

Peaks summed can be specified as only Named peaks, Only Unnamed peaks or Both.

The concentration of each individual peak in the group is calculated as described in section 11.4.

11.7 Limits of Detection (LOD) and Limits of Quantitation(LOQ)

The calculation of the LOD and LOQ for a compound is dependent upon the noise contained within the chromatogram trace used to quantify that compound.

11.7.1 Chromatogram Noise Calculation

Chromatogram noise is calculated as the standard deviation of the noise in the trace (effectively a height), which is multiplied by a user specified factor. Chromatogram noise may be calculated automatically or manually :

Manual Noise Measurement – To select this method, the user must specify an RT range of the trace which contains noise only. The resulting noise value is the standard deviation of the data in this region, multiplied by the User Factor.
Note : an RT entry of 0.0 may be used to signify the start or end of the trace when input as the start or end RT respectively.

$$Noise\ Height = SD_{selected\ data} \times User\ Factor$$

Where :

$$SD_x = \frac{\sum_N \left(x_i - Mean_x\right)}{N-1}$$

Automatic Noise Measurement – This method is initiated by selecting the entire range of the trace for noise measurement (i.e. start and end RT equal 0.0). An algorithm is used to determine which regions of the trace consist only of noise, the result being the standard deviation of these regions multiplied by the user specified factor.
Note : The noise detection algorithm relies on a relationship between the median difference between adjacent points and standard deviation which is specific to gaussian deviates. This approximation provides good results when used with raw chromatogram data, but not for smoothed data. As a result, the algorithm measures the noise in the raw trace, to which a scaling factor is then applied to produce the effect of the current smoothing parameters. This smoothing correction is specific to the effect of mean smoothing on gaussian deviates (see below). As a result, the accuracy of this method is affected when (a) Savitzky Golay smoothing is used and, to a lesser extent, when (b) the noise regions are not gaussian in nature.

$$SD_{\substack{mean \\ smoothed}} = \frac{SD_{raw\ data}}{Winsize^{0.5} \times Iterations^{0.25}}$$

Where :

$$Winsize = \left(2 \times WinHalfSize\right) + 1$$

This equation describes the relationship between the standard deviation of raw and mean smoothed data (for gaussian deviates). *Iterations* is the number of smooths applied to the raw data and *Winsize* is the size of the smoothing window. Masslynx requires a half window size to be entered which has the relationship with *Winsize* described in above.

A multiplication factor of 3 is commonly used as, for gaussian deviates, this corresponds to a range within which 95% of the data will lie.

11.7.2 Chromatogram Area Noise

The noise value is effectively a measure of noise height, an area value maybe calculated that is equivalent to the calculated noise. The Compound's IS peak is used for this, the ratio of height to area of the corresponding IS peak is calculated, this is applied to the noise value if the corresponding noise area is required.

11.7.3 Response Value for Noise

The absolute noise values are converted into equivalent response values.

If compounds are being quantified using Heights use the original noise values, if using Areas use the Area noise values.

Calculate an absolute noise response for the compounds based upon the method Secondary Parameters Compound response, Primary, Secondary or Both (in which case sum the noise values from both traces). If a Secondary trace is not specified the Primary is always used.

Calculate a noise response value from the absolute noise response by applying the IS response, in the same way as if it were a normal peak.

11.7.4 LOD and LOQ Concentrations

The LOD and LOQ concentrations are calculated by applying the compound's calibration curve to the noise response to obtain a value for the concentration, which is then multiplied by user specified factors for LOD and LOQ respectively. The LOD and LOQ factors are specified as part of the Quantify method and are set for all compounds, the default values are 3 and 8 respectively.

Resulting LOD and LOQ concentrations should be stored in Peak Record and be available for display in the Summary, if no value is available output should be blank.

11.7.5 LOD and LOQ Flags

These flags indicate if the calculated concentration of a compound falls below the LOD or LOQ concentration threshold. If a compound concentration exceeds the threshold the flag is 'Yes' if it is less than the threshold the flag is 'No'.