

Progenesis QI User Guide

Analysis workflow guidelines

Waters

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Introduction

This user guide takes you through a complete analysis of 22 LC-MS runs of metabolite containing samples for 3 biological groups (6 replicate runs per group) and a set of 4 QC runs using the unique Progenesis QI workflow. It starts with LC-MS data file loading then Alignment, followed by Analysis that creates a list of interesting compounds (molecules) which are identified with compound search and then explored within Compound Stats using multivariate statistical methods.

To allow ease of use the tutorial is designed to start with the restoration of an Archived experiment where the data files have already been loaded. However, the document covers all the stages in the Progenesis QI workflow, therefore if you are using your own data files please refer to Appendix 1 (page 79) then start at page 8.

How to use this document

You can print this user guide to help you work hands-on with the software. The complete user guide takes about 90 minutes and is divided into two sections. This means you can perform the first half focused on run alignment and analysis then complete the second half of analysis exploring comparative differences and compound identity at a convenient time. The table of contents allows you to focus on particular areas of the workflow.

Data used in this user guide

We would like to thank Dr Giorgis Isaac at Waters Corporation, Milford USA for providing the example data which has been adapted for this user guide as well as invaluable discussion on the handling of the data.

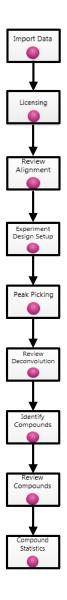
Workflow approach to data analysis

Progenesis QI adopts an intuitive Workflow approach to performing comparative LC-MS data analysis. The following user guide describes the various stages of this workflow (see below) focusing mainly on the stages from Alignment to Compound Stats.



Stage

. . .



Description	Page
Import Data: Selection and review of data files for analysis	7
Automatic Processing: setting up steps for automatic processing	8
After Automatic Processing: how to work with auto analysed data	12
Licensing : allows licensing of individual data files when there is no dongle attached (Appendix 3)	14
Review Alignment: automatic and manual run alignment	15
Experiment Design Setup: defining one or more group set ups for analysed aligned runs	22
Peak picking : setting parameters for and performing peak picking of compound ions	25
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Review Deconvolution : review and edit the various adduct, forms of a compound	30
Identify Compounds: search identity of compounds using Progenesis MetaScope and or other search engines	38
Review Compounds : managing possible compound identities exploring identity and expression between conditions	60
Compound Statistics : performing multivariate statistical analysis on tagged and selected groups of compounds	71

Restoring or starting a new Metabolomics Tutorial

If working with your original data files then refer to Appendix 1 page 79.

To use the Tutorial data:

Open Progenesis QI and download the DataSet for the LC-MS user guide (.zip) from the **'User guide and tutorial data**' link shown below, placing it in a **new folder** on your desktop. Before restoring the tutorial in the software **you must** first right click on the downloaded (.zip) file and extract it to a folder, i.e. 'C:\Users\john_smith\Documents\Progenesis QI Tutorial

QI Progenesis QI	
File Experiments	nonlinear
	A Waters Company
Recent experiments	Search Getting started Here are some resources to help you get started with Progenesis QI: • Eundamental concepts of the
QI Open Experiment	Progenesis QI workflow User guide and tutorial data Frequently-asked questions
Organize New folder	EZinfo version information
BitTorrent Sync Desktop Libraries Documents Music Prictures Videos Andy Borthwick Computer Network Control Panel Control Pan	Quickly go to an ion map location Want to quickly validate your sample running by zooming to a known ion?
	iments and Archives (*.Prc • pen • Cancel
Other experiments	Latest blog posts

Now you can restore the uncompressed Progenesis QI tutorial archive file. To do this, first locate the Tutorial Archive file using the **Open** button.

This opens the 'Import from archive' dialog.

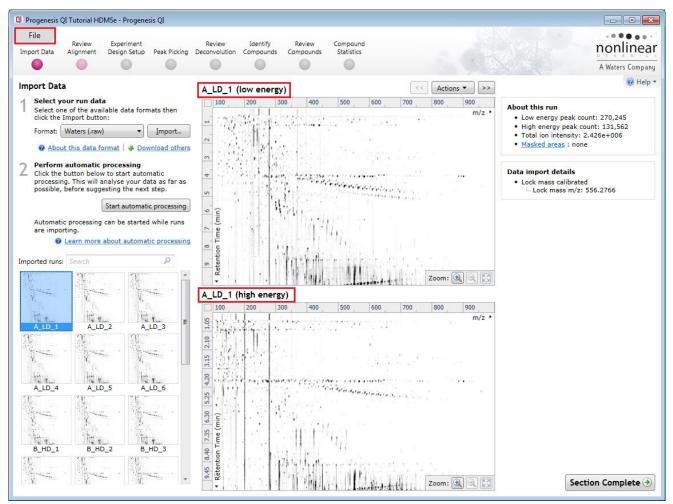
Select the **Create a new experiment** option and select the folder in which you placed the archive, using **Browse**.

QI Import Experiment fr	rom Archive			
Import experiment from archive After importing the experiment from this archive, any changes to the experiment will be saved to the location below, not back to the archive.				
 Replace an existin Experiment to replace Create a new experiment 	ace:			
Experiment name:	Progenesis QI Tutorial HDMSe			
Save to folder:	NProgenesis QI Tutorial_HDMSe\Progenesis Tutorial Browse			
	Import Cancel			

Then press Import.

Note: use the **Replace an existing experiment** option if you want to over-write an existing version of the tutorial.

The archive will restore with the software opening at the first stage in the workflow, Import Data. The panel on the left will display a 'thumbnail' for each run in the archive, indicating that they have been imported successfully.



The data file format for this tutorial was **.Raw**. It was acquired from a **SYNAPT-G2S** with the ionisation polarity set to **positive**.

You can look at the current **Experiment Properties** using the file menu. This displays details of the adduct definitions that were used during data import.

Note: total adduct abundances will be reported once Peak Picking has been performed.

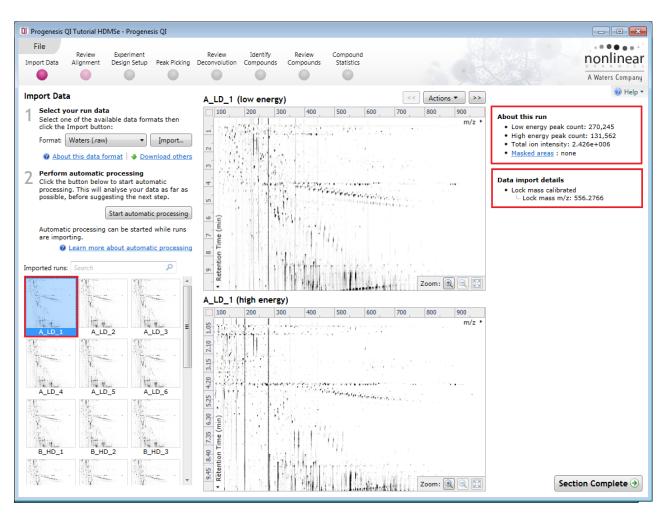
As this example is using HDMSe data it contains both low and high energy exact mass data.

The HD (High Definition) refers to the presence of Ion Mobility data (Drift Time)

Ionisation polarity: Positive Runs in this experiment: 22 Feature detection: High resolution Peak processing: Profile data				
Adduct definitions		iment:		
Name	Adduct Mass	Charge	Abundance	
M+2H	2.014552864	2		
M+H+Na	23.99649711	2		
M+H-2H ₂ O	-35.01385294	1		
M+H-H ₂ O	-17.0032882!	1		Ξ
M+H	1.007276432	1		
M+NH4	18.03382553	1		
M+Na	22.98922068	1		
M+CH₃OH+H	33.03349118	1		-

Stage 1: Data import and QC review of data set

The tutorial will now open at the Data Import stage (see below).



Each run appears as a 2D ion intensity map in the list. The current Run is displayed in the main window showing both low and high energy data. At this stage you will be warned if there are any data import errors for any of the files. The files will be highlighted in red and the error will appear to the right of the screen.

Note: if you have imported one or more runs that are either: not required for the experiment or are displaying data import errors (such as incorrect polarity) these runs can be removed by right clicking on the run in the list and selecting **Remove run**.

Details about the current run are displayed, top right, showing the Low and High energy peak counts and Lock mass calibration status.

Tip: the **'Mask areas for peak picking'** facility (under **Actions**) allows you to exclude areas (usually early and/or late in the LC dimension (Retention Time) that appear excessively noisy due to capture of data during column regeneration. This is **NOT** used for this data set.

Now click Start automatic processing.

Stage 2A: Automatic Processing of your data

The **Automatic Processing** of your data can be set up and started before the import of your data has been completed by clicking on **Start automatic processing.**

Note: for this tutorial the data has been imported already.



Setup of processing steps in the Analysis Workflow, up to and including Peak Picking, can be performed in the Automatic Processing wizard, these include:

- Select an alignment reference
- Automatic alignment of all runs to a reference run
- Define an Experiment design
- Automatic peak picking for compound ion detection

In this tutorial example you have 22 HDMSe LC-MS runs.

As the runs have already been imported, click **Start automatic processing** to setup the Automatic processing wizard.

Q Start automatic processing				
Select an alignment reference To compensate for drifts in retention time, all runs in the e to a single reference run.	experiment must be aligned			
How do you want to choose your alignment reference?				
Assess all runs in the experiment for suitability				
Use the most suitable run from candidates that I select				
🔘 Use this run:				
A_LD_1 *				
For information on choosing the alignment reference, and why you might want to select your own candidates, please see the <u>online guidance</u> .				
< Back	Next > Cancel			

Progenesis QI provides three methods for choosing the alignment reference run, as described below:

1. Assess all runs in the experiment for suitability

This method compares every run in your experiment to every other run for similarity, then selects the run with the greatest similarity to all other runs as the alignment reference.

If you have no prior knowledge about which of your runs would make a good reference, then this choice will normally produce a good alignment reference for you. This method, however, can take a long time for a large number of runs.

2. Use the most suitable run from candidates that I select

This method asks you to make a selection of reference candidates; the automatic algorithm then chooses the best reference from this subset of runs.

This method is appropriate when you have some prior knowledge of your runs suitability as a reference: i.e when all the candidate runs are pooled samples or when all the candidates are from a condition that displays the largest set of common peptide ions.

3. Use this run

This method allows you to manually choose the reference run.

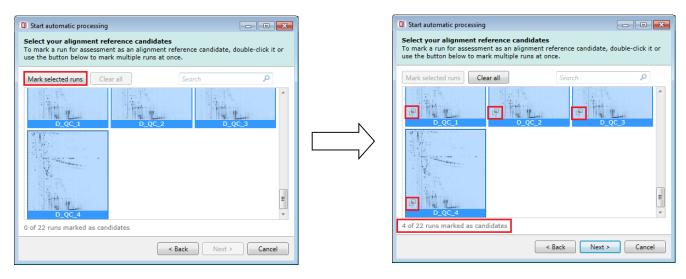
Manual selection gives you full control, but there are a couple of risks to note:

• If you choose a pending run which subsequently fails to load, alignment will not be performed.

• If you choose a run before it fully loads, and it turns out to have chromatography issues, alignment will be negatively affected (for this reason we recommend that you let your reference run fully load and assess its chromatography before loading further runs).

For this tutorial we will select the second option and click Next.

Now select all four of the QC runs as the Alignment Reference Candidates and click **Mark selected runs**. These will be marked with a Candidate icon. Now click **Next**.



You will now be asked if you want to align your runs automatically. Check box is ticked and select Next.

Automatic alignment After selecting the experiment's alignment reference, the software can also automatically align all runs. After the alignment reference is chosen, do you want to start automatic alignment? Ves, automatically align my runs
< Back Next > Cancel

The next page of the processing wizard will ask you to define an **Experiment Design.** To enter a design at this stage details of Experiment conditions must be available either as a MassLynx .SPL file or as a .CSV file.

Note: if these are not available leave the Set up option unticked and click next. You can enter a design(s) following automatic processing.

For this tutorial the .spl file **Progenesis QI_HDMSe Tutorial.spl** is available in the folder you restored the Tutorial Data Set.zip in.

0 Start automatic processing	QI Start automatic processing
Experiment design Experiment designs allow you to group and compare your samples according to their experimental conditions.	Experiment design Experiment designs allow you to group and compare your samples according to experimental conditions.
By defining an experiment design in advance, statistical measures such as ANOVA can be calculated automatically.	By defining an experiment design in advance, statistical measures such as ANOVA car calculated automatically. Set up an experiment design Enter a name for the experiment design: Conditions Load the criteria for grouping runs from this file: C:\Users\andy.borthwick\Documents\Customer Data\Progenesis QI v. Group runs by: VERSION VERSION Condition
What file formats are supported? Kext > Cancel	What file formats are supported?

Select the appropriate criteria by which to group by; in this example select 'Condition' and then click **Next** to move forward to the next page of the processing wizard.

This asks you if you want to **Perform peak picking** and allow you to **Set parameters**.

Q Start automatic processing
Peak picking Peak picking is the process by which we locate the compound ions and their isotopic peaks in your samples.
After the automatic alignment is finished, do you want to start automatic peak picking? Perform peak picking Set parameters
< Back Finish Cancel

For the purposes of this User guide we will use the default settings for peak picking.

Note: for more details on setting Peak Picking parameters refer to the section on Peak Picking (page 25).

Now click Finish to start the automatic processing.

The Alignment process starts with the automatic selection of **D_QC_4** as the reference

Q Automatic Processing	(23%)					
Automatic processi Current step: Choosing a	•					
Importing runs: Selecting reference: Aligning runs: Creating design: Peak picking:	22 of 22 processed Choosing an alignment reference Pending Pending Pending	Cancel	Autorr Current ✓ Impo Selec O Aligr Creat	matic Processing natic process step: Aligning 'A orting runs: ting reference: ning runs: ting design: picking:	ing	
						Cancel

Once Alignment completes the Design is applied and Peak Picking commences.

QI Automatic Processing (47%)	
Automatic processing Current step: Aligning 'B_HD_2'	
✓ Importing runs: 22 of 22 processed ✓ Selecting reference: D_QC_4 ✓ Aligning runs: 7 of 21 processed ✓ Control of the second secon	QI Automatic Processing Automatic processing Current step: Analysing
Creating design: Pending Peak picking: Pending	 ✓ Importing runs: 22 of 22 processed ✓ Selecting reference: D_QC_4 ✓ Aligning runs: 21 of 21 processed
Cancel	Creating design: Created Peak picking: Picking
	Cancel

Finally the Automatic Processing completes reporting the number of compound lons found.

Q Processing Complete Automatic process Time taken: 12 minutes of		×
 Importing runs: Selecting reference: Aligning runs: Creating design: 	22 of 22 processed D_QC_4 21 of 21 processed Created	
 Peak picking: 	6181 compound ions found	
		Close

As the whole process proceeds you get information on what stage has been performed and also the % of the process that has been completed.

Note: if Progenesis QI, during import of data and/or performing the various stages of Automatic Processing, detects problems with the data then the Processing dialog will report on the arising issue(s).

For example: failing to import one or more files.

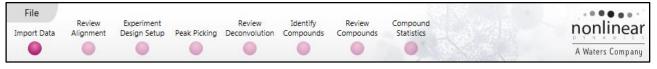
Details on the various warnings and managing failure messages are available in Appendix 2 (page 87)

Q Processing Complete			×
Automatic process	ing complete (with 21 seconds	warnings).	
A Importing runs:	22 of 22 processed 1 failed to import		
 Selecting reference: 	D_QC_3	•	
 Aligning runs: 	20 of 20 processed		
 Creating design: 	Created		
 Peak picking: 	6069 compound ions fo	ound	
			Close

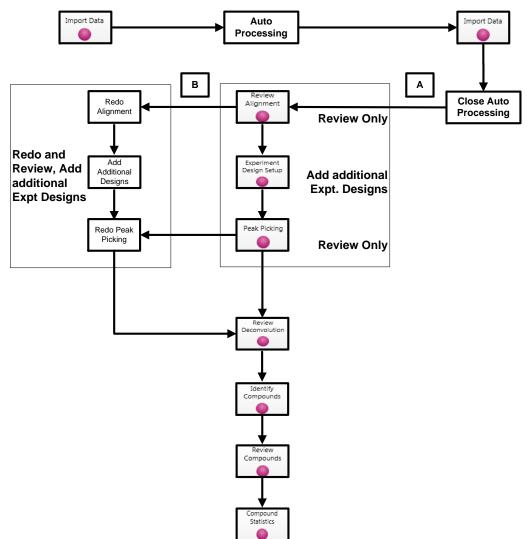
When the Automatic Processing completes click **Close**, the software remains at the Import Data stage of the workflow where you can review the chromatography of the imported runs and remove/replace any runs that have failed to import.

Stage 2B: After Automatic Processing

When Processing completes, access to the later steps in the analysis workflow will depend on the steps performed in the automatic processing. Here we outline the various paths and options you can take to proceed with the analysis following Automatic Processing.



The flow chart gives you an overview of the various steps you can take to proceed with your automatically processed data.



In this example where the data has been automatically processed once you **Close** the completed automatic processing dialog you can either:

- **Option A**: Review the Chromatography at the Import Data stage (page 13) and then continue with Reviewing Alignment quality (page 15).
- **Option B**: During the review process you decide to redo the alignment (page 15) and/or Peak Picking (page 25).

Note: the tutorial data described here does not require redoing either alignment or peak picking so you can step straight to Review Deconvolution. However, this guide is designed to support the analysis of your own data sets as well, so it covers all stages of the workflow.

Tip: it is good practice to close your experiment following automatic analysis and create an archive (see FAQs for details on creating an archive), and then reopen it and continue with analysis.

Review Chromatography

Each data file appears as a 2D representation of the run. In the case of MSe and HDMSe data both high and low energy maps are displayed.

At this stage you will be warned if any of the data files have failed to import. The reasons for import failure(s) include: run is centroided when importer is expecting a profile (continuum) run, the run has a different polarity to the other runs etc.

You can delete selected run(s) by left clicking on the run(s) in the list.

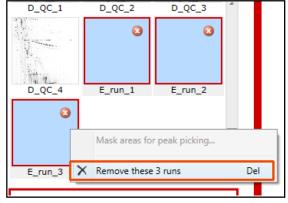
Note: for this data set do not remove any files

You can examine the quality of the imported runs using the 2D representation of the runs making use of the Zoom tools (bottom right of the ion maps) and Panning tools.



Note: details of the current run appear on the top right of the view.

Once you have reviewed the imported runs click on **Review Alignment** or **Section Complete** to move forward to the Review Alignment Stage.



Stage 3: Licensing

This stage in the analysis workflow will **only** appear if you are using 'Unlicensed' data files to evaluate the software and have no dongle attached.

For details on how to use Licensing go to Appendix 3 (page 90)



If you are using the tutorial archive, this page will not appear as the data files are licensed.

If you have performed an analysis using Automatic Processing without a valid dongle or do not have the appropriate code to licence your runs, if you close Progenesis QI you will be warned that the analysis will be lost.

File nport Data	Licensing	Review Alignment	Experiment Design Setup	Peak Picking	Review Deconvoluti	Identify on Compounds	Review Compounds	Compound Statistics			nonli	nea
								0			A Waters C	ompan
	cense Runs											
This installa icensed rui	ation is curren ns only.	tly restricted	to analyse	Run name						Licence state	License this run	^
Lo liconeo :	your runs, you	u need an ev	valuation or	C:\Users\andy	.borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	\checkmark	
	ce code whic			C:\Users\andy	.borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	V	
a sales rep	resentative.			C:\Users\andy	.borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	\checkmark	
Once licens	ed, your runs	s can be ana	lysed on	C:\Users\andy	.borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	V	
any installa	tion of the sof	ftware. The li	cence is	C:\Users\andy	.borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	V	
	lly included w	hen archivin/	g an	C:\Users\andy	borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	V	
experiment				C:\Users\ar	📙 Unable to	save experimen	t		×	QI T Unlicensed	V	
	have been lic lick here to m			C:\Users\ar						QI T Unlicensed	V	
available on	this compute	er.		C:\Users\ar			analysis withou	ut a valid license	If you close now your	QI T Unlicensed	V	Ξ
f vou have	one, you can	open a licenc	e file to	C:\Users\ar	anal	ysis will be lost.				QI T Unlicensed	V	
nstall.				C:\Users\ar						QI T Unlicensed	v	
f vou have	just installed	a donale, clic	k here	C:\Users\ar						QI T Unlicensed	V	
.,	,			C:\Users\ar				0	K Cancel	QI T Unlicensed	V	
				C:\Users\andy	borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	v	
				C:\Users\andy	.borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	V	
				C:\Users\andy	.borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	V	
				C:\Users\andy	borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	v	
				C:\Users\andy	borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	V	
				C:\Users\andy	.borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	\checkmark	
				C:\Users\andy	.borthwick\D	ocuments\Custom	er Data\Progene	sis QI v2.2_Demo	Suites_Tutorial\Progenesi	s QI T Unlicensed	\checkmark	-
							Run licence	code:	·	Use L	icence Cod	e
											tion Comple	

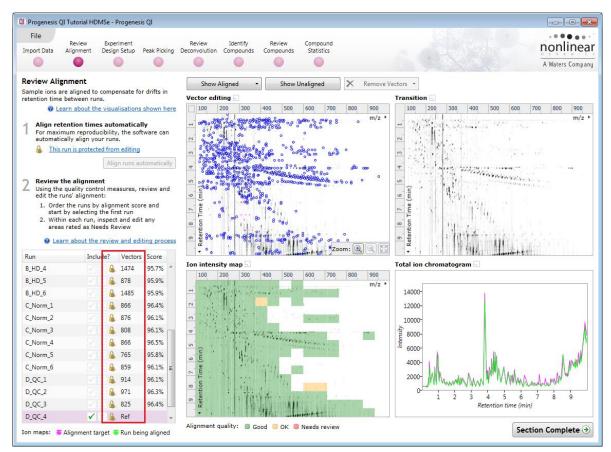
Note: although the analysis will be lost (Alignment Peak Picking etc) the experiment file and all the 'loaded pre-processed runs are retained'. This allows you to reopen the experiment, when you have a licenced code and/or dongle available, and redo the analysis steps without having to reload the data.

For more details on managing the licencing of your data refer to Appendix 3 (page 90)

Stage 4: Review Alignment

At this stage, **Review Alignment** opens displaying the alignment of the runs to the Reference run (D_QC_4).

Having performed the analysis automatically there will be icons next to each run in the vectors table indicating that the run is protected from editing.



Note: for this tutorial data set, refinement of the alignment is **NOT** required, so you can move to page 19 without altering the alignment.

However, when reviewing the quality of alignment after 'Automatic Analysis' of a data set you may decide that the alignment requires editing. Reviewing and editing are described in the following sections.

To do this click on the **This run is protected from editing** link above the 'greyed out' **Align runs automatically** button.

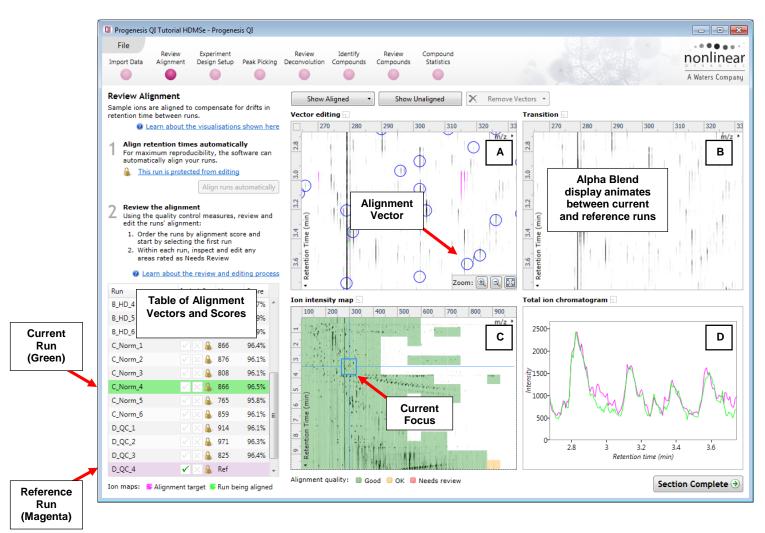
Review Alignment Sample ions are aligned to compensate for drifts in retention time between runs.	Q Protected from editing
Align retention times automatically For maximum reproducibility, the software can automatically align your runs. Image: This run is protected from editing Align runs automatically	Delete existing analysis? If you change the alignment, it will invalidate the current analysis including compound ion pattern and IDs, editing, and tags. If you want to keep these, you should archive this experiment before changing the alignment and moving forward.
 Review the alignment Using the quality control measures, review and edit the runs' alignment: Order the runs by alignment score and start by selecting the first run Within each run, inspect and edit any areas rated as Needs Review Learn about the review and editing process 	Delete analysis and allow editing Cancel

Note: as you click on the link, you will be warned that you are discarding the current analysis (all steps beyond alignment).

Details on editing alignment are described in Appendix 4 (page 91).

Layout of Alignment

To familiarize you with Progenesis QI Alignment, this section describes how to use the various graphical features used in the alignment of the LC-MS runs.



Vector Editing (Window A): is the main alignment area and displays the area defined by the current **focus** rectangle shown in Window C. The current run is displayed in green and the reference run is displayed in magenta. Here is where you can review in detail the vectors and also place the manual alignment vectors when required.

Transition (Window B): uses an **alpha blend** to animate between the current and reference runs. Before the runs are aligned, the features appear to move up and down. Once correctly aligned, they will appear to pulse. During the process of adding vectors, this view can be used to zoom in and also navigate thus helping with accurate placement of manual vectors.

Ion Intensity Map (Window C): shows the **focus** for the other windows. When you click on the view the blue rectangle will move to the selected area. The focus can be moved systematically across the view using the cursor keys. The focus area size can be altered by clicking and dragging out a new area with the mouse. This view also provides a visual quality metric for the Alignment of the runs, from green through yellow to red.

Total Ion Chromatograms (Window D): shows the current **total ion** chromatogram (green) overlaid on the Reference chromatogram (magenta). As the features are aligned in the **Vector Editing** view the chromatograms become aligned. The retention time range displayed is the vertical dimension of the Focus Grid currently displayed in the **Ion Intensity map** (Window C).

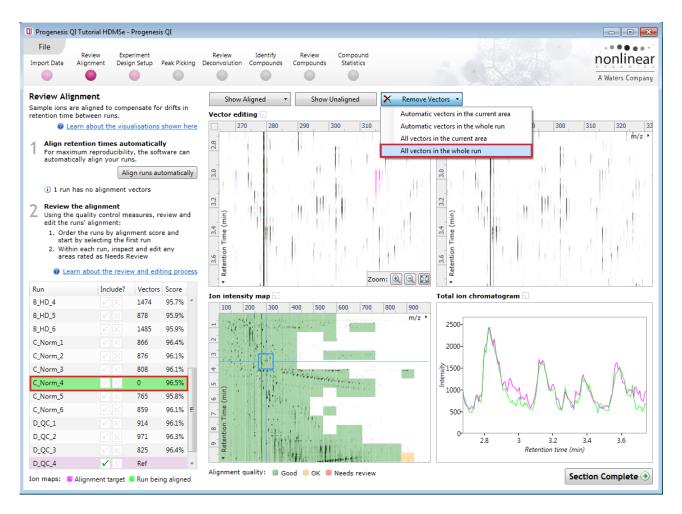
To best understand the process of alignment and the meaning of the scoring we will now undo the automatic alignment for C_Norm_4, then simulate a poorly aligned run by adding an incorrect manual vector.

To setup the display so that it looks similar to the one below:

- If you have not already unlocked the Analysis (note padlock icons in the runs table see right) then refer to page 15.
- In the Run table click on C_Norm_4 to make it current. You will now be looking at the alignment of C_Norm_4 to D_QC_4 in the Unaligned view.

Run	Include?	Vectors	Score	
B_HD_3	🗹 🗙 🔒	826	96.0%	*
B_HD_4	$\checkmark \times \clubsuit$	1474	95.7%	
B_HD_5	🗹 🗙 🌡	878	95.9%	
B_HD_6	$\checkmark \times \clubsuit$	1485	95.9%	
C_Norm_1	$\mathbb{Z}\times\mathbb{A}$	866	96.4%	
C_Norm_2	$\checkmark \times \clubsuit$	876	96.1%	
C_Norm_3	$\boxtimes \times \clubsuit$	808	96.1%	
C_Norm_4	🗸 🗙 🌡	866	96.5%	
C_Norm_5	X × 4	765	95.8%	
C_Norm_6	$\checkmark \times \clubsuit$	859	96.1%	Ξ
D_QC_1	$\mathbb{Z}\times\mathbb{A}$	914	96.1%	
D_QC_2	$\checkmark \times \clubsuit$	971	96.3%	
D_QC_3	$\swarrow \times \clubsuit$	825	96.4%	
D_QC_4	🖌 🖂 🌡	Ref		-

First click on **Remove Vectors** and then select remove All vectors in the whole run.

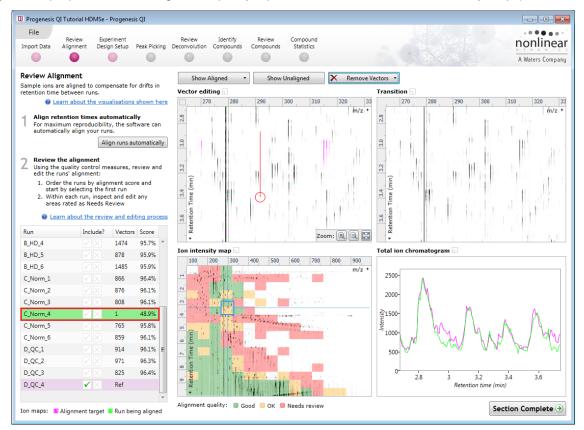


Note: the Retention Time alignment of this data is good between the runs as indicated by a high percentage score (in the absence of any vectors) and the Alignment quality showing as 'all' green.

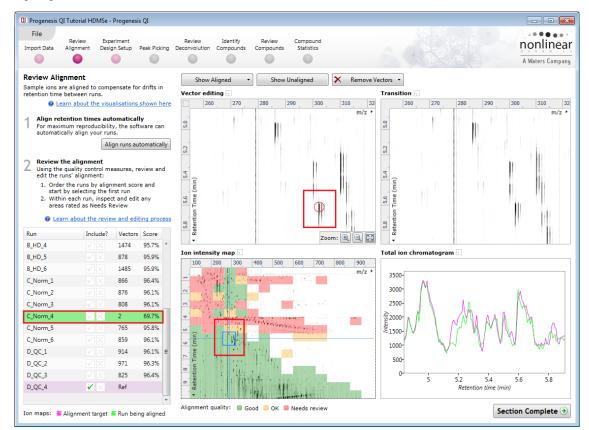
To simulate poor alignment, place a single manual vector on the Vector editing view (Window A). To do this, in the Vector Editing panel, click and drag out a single vector then release the mouse button. By doing this a single manual vector will appear with a length corresponding to the 'drag'.

Note: the manual vector is red, to distinguish it from the automatic vectors (blue).

The effect of adding this incorrect manual vector is to markedly reduce the Alignment score and also cause a significant proportion of the Alignment quality squares to turn red on the Ion Intensity Map (as shown below).



Now place a second vector at a different location **BUT** this time click and release **without** deliberately misaligning the vector.



Notice how the correctly placed vector markedly improves the Score this is also reflected in the increased proportion of the Ion Intensity Map showing green indicating a good quality alignment.

Reviewing quality of alignment vectors

After **Automatic alignment** the number of vectors and Quality Scores will be updated on the **Runs** panel and the vectors will appear (in blue) on the view.

Where the alignment has worked well, the alignment views will look as below with the Ion Intensity Map showing green indicating good quality alignment and the Transition view showing features pulsing slightly but not moving up and down.



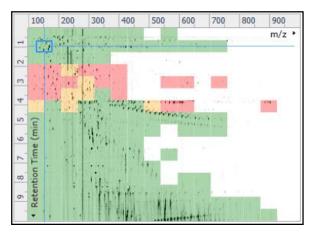
Using the **Simulated** miss-aligned example from the previous section to explain the review process for alignment, the alignment looks as below with a region of poor alignment (highlighted in red).

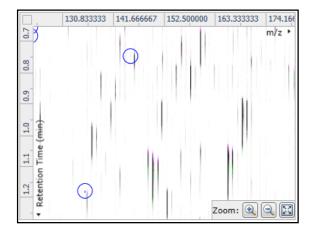


Reviewing Quality of Alignment

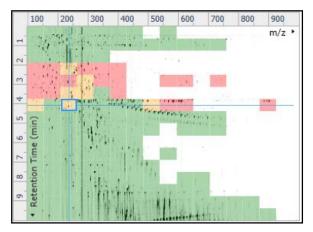
At this point the quality metric, overlaid on the Ion Intensity Map as coloured squares, acts as a guide drawing your attention to areas of the alignment. These range from Good (Green) through OK (Yellow) to Needs review (Red). Drag out a 'Focus' area that corresponds to one of the coloured squares. Three example squares are examined here.

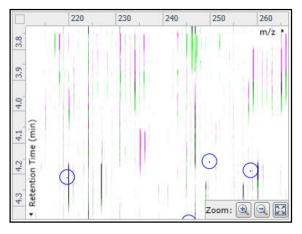
For a 'green' square the majority of the data appears overlapped (black) indicating good alignment. When viewed in the Transition view the data appears to pulse.



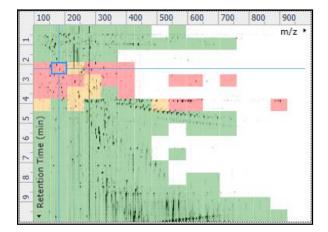


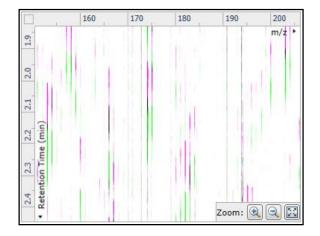
For a 'yellow' square some of the data appears overlapped (black) indicating OK alignment. When viewed in the Transition view some of the data appears to pulse.





For a 'red' square, little if any of the data appears overlapped (black) indicating questionable alignment.

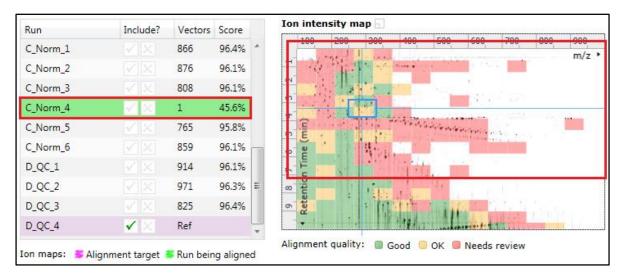




When viewed in the Transition view little data appears to pulse.

Note: the coloured metric **should be used as a guide**. In cases where there are a few 'isolated' red squares this can also be indicative of 'real' differences between the two runs being aligned and should be considered when examining the overall score and surrounding squares in the current alignment.

The weighted average of the individual squares gives the overall percentage score for each run's alignment.



Note: a marked red area combined with a low score clearly indicates a 'miss alignment' and may require some manual intervention (see Appendix 4, page 91).



The alignment quality of this data set does not require any manual intervention so before going to the next section make sure you have Removed all manual vectors and re-performed the Automatic alignment. To do this for C_Norm_4 first select Remove 'All vectors in the whole run' and then click Align runs automatically.

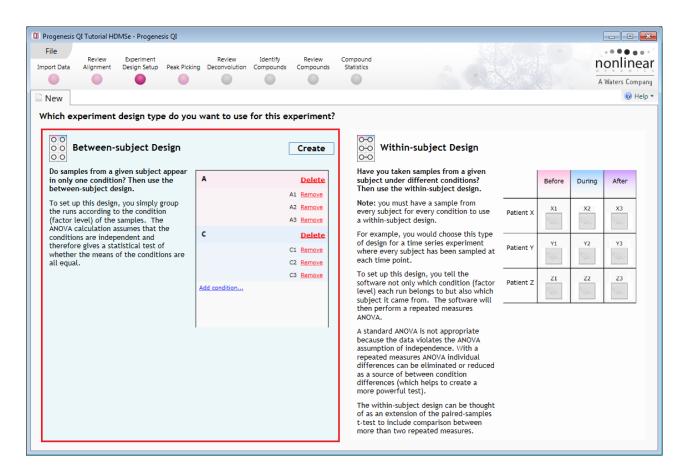
Having aligned the runs automatically click **Section Complete** to move to Experiment Design Setup.

Stage 5: Experiment Design Setup for Analysed Runs

At this stage in the workflow you can setup one or more experimental designs for your data.

Note: If you have created a design as part of the automatic processing then it will appear as a Design tab. However if you have reperformed the analysis 'manually' then the only tab displayed will be **New**.

There are two basic types of experimental designs:



Between-subject design: here samples from any given subject appear in only one condition. (i.e. control versus various drug treatments). The ANOVA calculation assumes that the conditions are independent and applies the statistical test that assumes the means of the conditions are equal.

Within-subject design: here samples have been taken from a given subject under different conditions (i.e. the same subject has been sampled over a period of time or after one or more treatments). Here a standard ANOVA is not appropriate as the data violates the ANOVA assumption of independance. Therefore by using a *repeated measures* ANOVA, individual differences can be eliminated or reduced as a source of between condition differences. This within-subject design can be thought of as a extension of the paired samples t-test, including comparison between more than two repeated measures.

Additional information on how to apply the Within-subject Design is in Appendix 5 page 96

This experiment contains 4 conditions: A, B, C and D and uses the **Between-subject design** to group the analysed runs to reflect the Biological conditions in the original study.

To create a new **Between-subject Design** move the cursor over this option and click to open the dialog.

Q Create New Experiment Design	×
Enter a name for the experiment	design:
All conditions	(
How do you want to group the ru	ns?
Group the runs manually	
Copy an existing design:	Ψ
Import criteria from a file:	Browse
Group runs by:	no valid groups>
What file formats are supported	? Create design Cancel

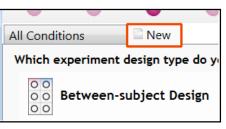
Give the new experimental design a name and then click Create design.

QI Progenesis QI Tutorial HDMSe - Progenesis QI				
File Review Experiment Import Data Alignment Design Setup Peak Picking	Review Identify Review Deconvolution Compounds Compounds	Compound Statistics		A Waters Company
All conditions I × New				🔞 Help 🔻
Setup conditions	Runs Add Selected Runs to Condition	▼ Search	Q	
Setup the conditions that you want to compare below (e.g., control, drug A, etc), and then assign each of your samples to the correct condition.	Add to new condition	B_HD_2	B_HD_3	^ _
C_Norm Delete	C_Norm	200	5.44	
C_Norm_1 Remove	A_LD			
C_Norm_2 Remove				
C_Norm_3 <u>Remove</u> C_Norm_4 <u>Remove</u>	B_HD_4	B_HD_5	B_HD_6	
C_Norm_5 Remove	Sec.	S. H.	234 C.	
C_Norm_6 Remove				
A_LD Delete				=
A_LD_1 Remove	D_QC_1	D_QC_2	D_QC_3	
A_LD_2 Remove		and the second	and the second se	
A_LD_4 Remove				
A_LD_5 Remove	D_QC_4			
A_LD_6 Remove				
Add condition				
				*
			S	ection Complete 🏵

To create a new condition

- 1. Select the runs for the condition by clicking on the required icon in the **Runs** panel, as shown.
- 2. Press the 'black triangle' next to the Add Selected Runs to Condition button on the main toolbar.
- 3. Select Add to new condition... from the drop down menu.
- 4. A new condition will appear in the **Conditions** panel on the left.
- 5. Rename the condition (e.g. B_HD) by over typing the default name.
- 6. Repeat steps 1 to 5 until all the required runs are grouped into conditions.

An alternative way to handling the grouping of this set and other larger (and more complex) experimental designs is to make use of **sample tracking information** that has been stored in a spread sheet at the time of sample collection and/or preparation.



For this example there is a **Progenesis QI_HDMSe Tutorial.SPL** file available in the Experiment Archive you restored at the beginning of this tutorial exercise.

To use this approach select the **Import design from file** option from the New Experiment Design dialog. Then locate the Tutorial Groups file and select what to **Group runs by**, for example: Condition.

Q Create New Experiment Desig	n	
Enter a name for the experime	ent design:	
Minus QC		
How do you want to group the	e runs?	
\bigcirc Group the runs manually		
Copy an existing design:	All conditions	~
Import criteria from a file:	genesis QI_HDMSe Tutorial.spl	Browse
Group runs by:	Condition	•
	VERSION	-
What file formats are support	Condition	
what he formats are suppor	Index	

When **Create design** is pressed the new tab refreshes to allow you to adjust the conditions.

Use **Delete** on the Conditions panel to remove conditions that are not required in this particular design i.e in this example the **QC**.

QI Progenesis QI Tutorial HD	MSe - Progenesis QI		
File Import Data Alignment	Experiment Design Setup Peak Picking	Review Identify Review Compound Deconvolution Compounds Compounds Statistics	A Waters Company
All conditions	Minus QC I 🗙 📄	New	🛞 Help 🔻
Setup conditions Setup the conditions that (e.g., control, drug A, etc) your samples to the correct	you want to compare below), and then assign each of ct condition.	Runs Add Selected Runs to Condition	٩
	A_LD_6 Remove		
B_HD	Delete		
	B_HD_1 Remove		
	B_HD_2 Remove		
	B_HD_3 Remove		
	B_HD_4 <u>Remove</u>		
	B_HD_5 Remove		
o c	B_HD_6 Remove		
<u> </u>	Delete		
	D_QC_1 Remove		
	D_QC_2 <u>Remove</u>		
	D_QC_4 Remove		
Add condition			Section Complete $ e e e e e e e e e e e e e $

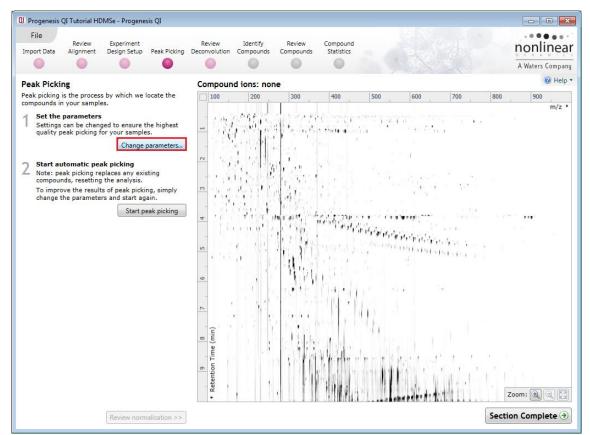
Note: On deleting a condition the runs will reappear in the Runs window.

Note: both designs are available as separate tabs.

To move to the next stage in the workflow, Peak Picking, click Section Complete.

Stage 6A: Peak Picking

Having set up one or more Experiment Designs for your data the Peak Picking stage will open as shown below. Here you can define thresholds for the detection of peaks across all of your aligned runs.



Note: if you have processed your data automatically and have NOT discarded your analysis as described on page 15 then the Peak picking will appear as shown on page 27.

The ion intensity map displayed before Peak picking is for the Alignment Reference run. As the Peak Picking takes place this is replaced by the Aggregate map generated from all the aligned runs in your experiment.

Peak Picking Parameters

The Peak Picking Parameters dialog opens by clicking on **Change parameters...** showing all the runs in the current experiment and a tick against each run. This is the default setting, where the peak picking algorithm uses information from all of the runs to contribute to the pattern of compound ion outlines.

Runs for peak picking	Peak picking limi	ts Retention time	e limits	
Choose runs for p	eak picking -			
You can tick or un-tic		🗷 Run		
control which will be used by the beak picking algorithm. Although any run which is left un-ticked will not affect the compound ion outlines, it	A_LD_1			
	A_LD_2			
will still have outlines		A_LD_3		
design setup.	available in the experiment setup.	A_LD_4		
Learn more about why you might not want to select all runs.	A_LD_5			
	A_LD_6		=	
	B_HD_1		1	
		B_HD_2		
		B_HD_3		
		B_HD_4 B HD 5		
		B HD 6		
		C Norm 1		
		C Norm 2		
		C_Norm_3		
		C Norm 4		
		C Norm 5		-

Q Peak Picking Parameters	×
Runs for peak picking Peak picking li	mits Retention time limits
Sensitivity You can adjust the sensitivity of	
the peak picking algorithm using these different methods. Each	Automatic Absolute ion intensity
sensitivity method examines the intensities of groups of MS peaks to judge whether they are likely to	🔘 % Base Peak
form part of an ion or whether they represent noise and so should be ignored. Peaks that are rejected as noise will not be used to build ion outlines.	The automatic sensitivity method uses a noise estimation algorithm to determine the noise levels in the data. The higher the sensitivity value, the more compound ions will be detected.
	fewer default more
Chromatographic peak width	
The chromatographic peak width gives the length of time over which an ion has eluted. If you set	Apply a minimum peak width
a minimum peak width, any ion that has eluted over a shorter period will be rejected.	Minimum width: 0 minutes
	OK Cancel

Tip: It may be appropriate **only** to pick peaks that are present in a limited number of your runs. In which case un-tick the runs that you do **NOT** want to contribute to the feature detection pattern. This may be important when one or more of the runs appear noisy due to non-optimal chromatography or sample handling.

Note: feature outlines will be added to 'un-ticked' runs; however, these runs will not contribute to the peak picking pattern.

Tip: depending on run quality, a suggested minimum number of ticked runs should include at least one replicate of each experimental condition.

The sensitivity of the peak picking can be controlled by adjusting settings under the Peak picking limits tab.

Note: for all 3 **Sensitivity** modes a Chromatographic peak width (Retention time window) for the peaks **can** be set by applying a minimum retention window or peak width in minutes.

Chromatographic peak width			
The chromatographic peak width gives the length of time over which an ion has eluted. If you set	Apply a minimur	n peak wid	lth
a minimum peak width, any ion that has eluted over a shorter	Minimum width:	0.15	minutes
period will be rejected.			

For the runs in this user guide, we will use the default settings for the Automatic method and NOT apply a minimum peak width (as shown above).

Finally you can set **Retention time limits** for the detection. The default limits are displayed if no limits are defined.

Runs for peak picking	Peak picking limit	s Retention time limi	ts
Retention time li			
You can set the minim maximum retention ti picking. Ions that elut after these values will	num and me for peak e before or	Ignore ions before Ignore ions after	9.9972833: minutes

Click **OK** to close the Peak Picking Parameters dialog.

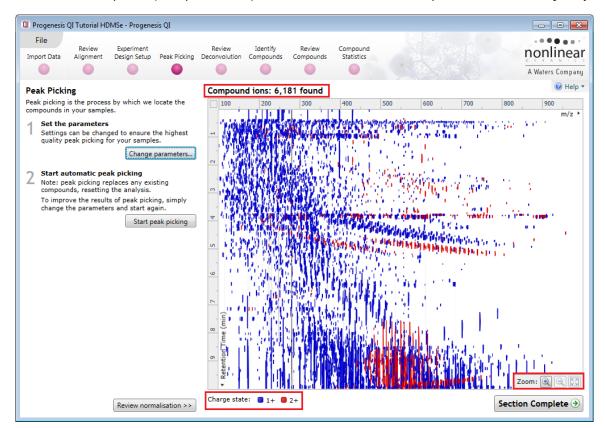
Press Start peak picking to start the detection process.

Analysing		

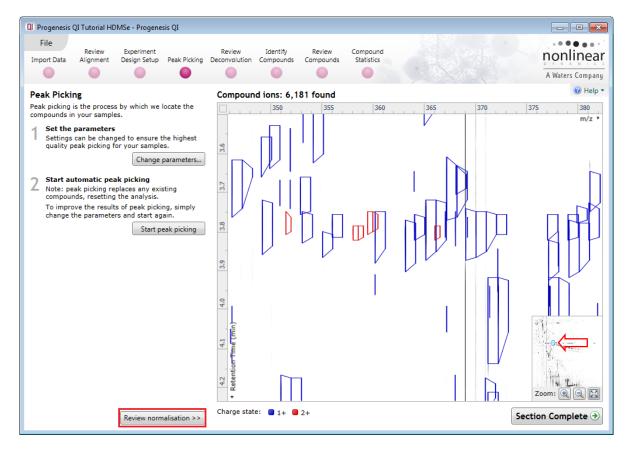
During peak picking, a progress bar will appear telling you that it is Analysing.

Following automatic peak picking the detected peaks are displayed in a colour according to their charge state. (note: number of charge states represented reflects Adduct Definitions used in the experiment)

The actual number of peaks (Compound Ions) detected is recorded at the top of the ion intensity map.



To inspect the detection more closely drag out an area of interest with the zoom tool.



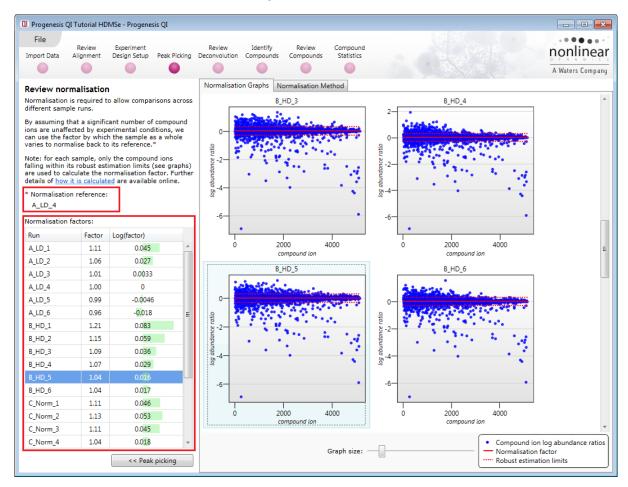
The normalisation of the data can be reviewed by clicking on Review normalisation.

Stage 6B: Reviewing Normalisation

The **Review Normalisation** page will open displaying plots for the normalisation of all the peaks on each run.

This page in the workflow **does not** allow you to alter the Normalisation of your data but provides you with individual views for each run showing the data points used in the calculation of the normalisation factor for the run.

The default method is to Normalise to all compounds.



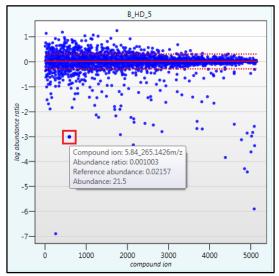
Normalisation factors are reported in the table to the left of the plots.

Calculation of Normalisation Factor:

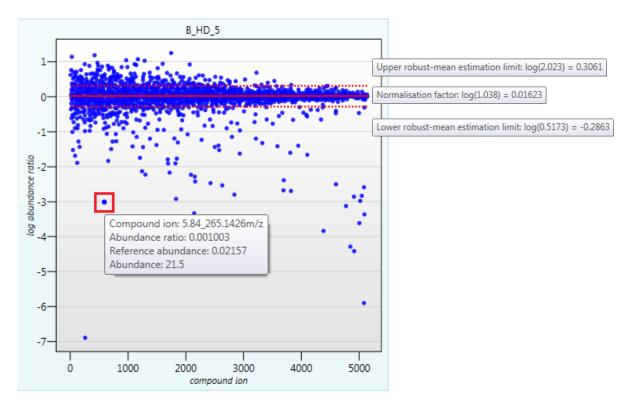
Progenesis QI will automatically select one of the runs that is 'least different' from all the other runs in the data set to be the 'Normalising reference'. The run used is shown above the table of Normalisation factors (in this example it is A_LD_4).

For each sample run, each blue dot shows the log of the abundance ratio for a different compound (normalisation target abundance/run abundance).

The details for individual Compound ions can be viewed as you hold the cursor over the dots on the plot. On the graph the compounds are shown ordered by ascending mean abundance.



The normalisation factor is then calculated by finding the mean of the log abundance ratios of the compounds that fall within the 'robust estimated limits' (dotted red lines). Compounds outside these limits are considered to be outliers and therefore will not affect the calculation of the normalisation factor.



Finally, if you do not wish to work with normalised data then you can **use the raw abundances** by switching off the normalisation.

Note: there are 5 Normalisation modes

Review Deconvolution	Identify Compounds	Review Compounds	Compound Statistics	nonlinear
			•	A Waters Company
Normalisatio	on Graphs No	ormalisation Me	ethod	
Normalise to	o all compound	is 🔻		
Normalise to	o all compound	ls		
Normalise u Normalise to	o a set of house sing total ion a o external stand ny normalisatio	dard	pounds	

Note: once you have identified a set of 'housekeeping' compounds you can then apply the Normalise to a set of housekeeping compounds by using this option to locate and select the compounds.

For this example experiment, you should leave the Normalise to all compounds option selected.

Now return to **Peak picking** by clicking on the button on the bottom left of the screen and the press **Section Complete** to move to the Review Deconvolution stage of the workflow.

Stage 7: Review Deconvolution

Following Peak Picking all the ions, including various adducts, for a compound are automatically recombined to provide accurate quantitation of each parent compound. Adducts of the same compound are grouped by a process called deconvolution. This stage of the workflow allows you not only to review the outcome of the deconvolution but also to add or remove adduct forms of the compound.



At this stage in the workflow you get the opportunity to review the process of Deconvolution for the Compounds. The table on the left displays the compounds ordered by the number of Adducts detected for each compound.

Note: the detected adducts are dependant on the list of expected adducts that you selected when you created the experiment

The total number of expected adducts is displayed as a montage at the top of the screen.

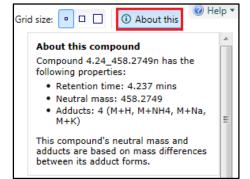
For an example: In **Compound 4.24_458.2749n**, 4 adducts have been detected: M+H, M+NH₄, M+Na and M+K (arrows) and displayed in the table. To find this compound easily, one may order the table by compound using the header or scroll down slightly on the Compounds table.

Tip: the 'About this' panel, top right summarises the information for the current compound. If not displayed click on the About this.

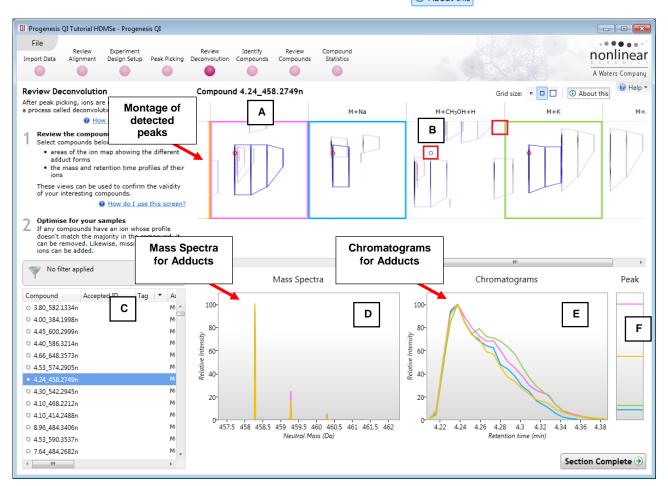
The same details are available for the compound in the table to the bottom left.

Use the Grid size to enlarge the montage view of the adducts.

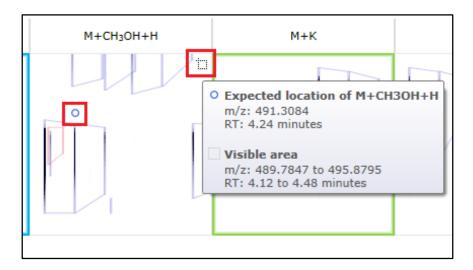
Using **Compound 4.24_458.2749n** we can look at how the information is displayed on the multiple views following deconvolution.



Note: for clarity the 'About this' pane can be hidden by clicking on (i) About this



- (A) Montage view showing location of detected adducts for compound
- (B) Where an adduct has not been detected the expected location for the adduct is displayed on the relevant panel as a blue circle when you hover the cursor over the panel. In this case no Methanol adduct (M+CH₃OH + H) has been detected.

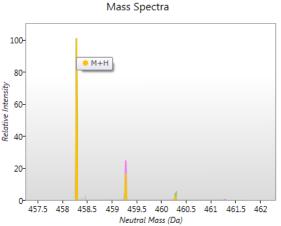


Note: hover the cursor over the icon on the top right of the panel and you will get information about the expected location and the area displayed.

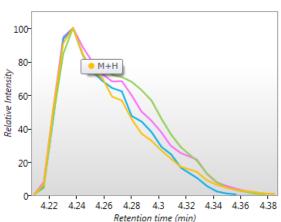
(C) The table displays the information known about the current compound with regards to the number of adducts, identity of the adducts and where deconvolution has been successful the Compound's Neutral mass is displayed. Note: when you hover the cursor over the Neutral mass for the current compound it displays the adducts and their m/z values that were used to generate the the value. This is shown in an expanded view of the table below. Also shown is the 'tool tip' for the displayed m/z for each compound. An example is shown below for Compound 4.24_458.2749n.

Compound	Accepted ID	Tag 🔹	Adducts	Adduct count	Neutral mass	m/z Retention time Fragmented?	
0 4.66_648.3573n			M+H, M+NH4, M+Na, M+K	4	648.3573	666. 2011	
O 4.53_574.2905n			M+H, M+NH4, M+Na, M+K	4	574.2905	592. The m/z of the most abundant compound	
• 4.24_458.2749n			M+H, M+NH4, M+Na, M+K	4	458.2749	476.: ion. To calculate this, each ion's abundance is defined as the sum of un-normalized	
O 4.30_542.2945n				4	542.2945	560. abundances for that ion across all samples.	
O 4.10_468.2212n			M+H, M+ M+H at m/z=459.2803	4	468.2212	486.2550 4.097 Yes	
O 4.10_414.2488n			M+H, M+ • M+NH ₄ at m/z=476.3087	4	414.2488	432.2827 4.104 Yes	
O 8.96_484.3406n			 M+H, M+ M+Na at m/z=481.2619 M+K at m/z=497.2361 	4	484.3406	502.3745 8.961 Yes	
O 4.53_590.3537n			M+H, M+	4	590.3537	608.3875 4.526 Yes	

(D) This panel shows the Mass Spectra for the detected adducts of the Compound, colour coded by adduct, where the relative intensities are plotted against the **Neutral mass** scale.



Chromatograms



(E) This panel shows the Chromatograms for the detected adducts of the Compound, colour coded by adduct, where the relative intensities are plotted against the **Retention Time** scale.

Peak

• M+H

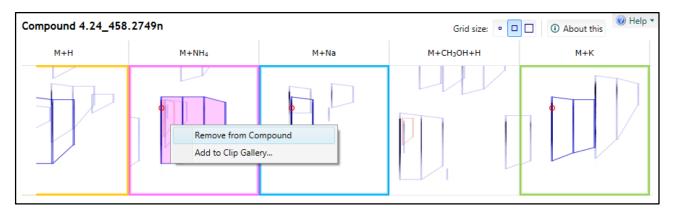
(F) This panel shows the actual Peak heights for the detected adducts of the Compound, colour coded by adduct.

Editing Compound adducts.

The number of adducts assigned to a compound by the process of deconvolution depends on the peak detection and the adducts selected at the beginning of the workflow when the experiment is created.

The process of deconvolution can only assign a Neutral mass for a compound if two or more adducts have been detected. The accurate quantitation of a compound is dependent on summing the intensities for a compounds adducts. In addition the accuracy of the quantitation for any compound is dependent not only on the detection of the adducts but also on the correct assignment during the process of deconvolution. In a complex sample there may be a need to add or remove adducts from a compound.

To remove an adduct right click on the assigned peak in the appropriate panel of the montage. So as an example we require to remove the $M+NH_4$ adduct for compound **Compound 4.24_458.2749n**, right click on the peak in the adduct panel and click **Remove from compound**.



In the table the number of adducts for the compound is reduced by 1 and a tag (grey) indicating that the adducts of this compound have been edited is created.



To add an adduct to a compound right click on a peak in the appropriate panel for the adduct and click **Add to compound**, again the table of compounds will update to reflect the change.

If you now remove 2 of the remaining 3 adducts the montage view will change to display the full matrix of all possible peak locations for expected second adducts dependant on the m/z values for these adducts. Also as there is only one adduct detected the Neutral mass for the compound can no longer be calculated.

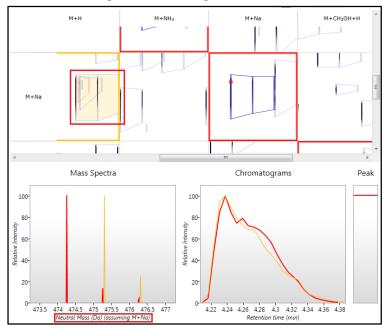
Try removing all the adducts from Compound 4.24_458.2749n to leave just the M+K.

The montage now turns into a matrix displaying all the possible locations for a charge state +1 Adduct in accordance with the list of expected adducts you selected at the beginning of the experiment. Also the individual Compound ions are displayed in the table i.e. **Compound 4.24_497.2361**

Note: because the peak is a charge state +1 this excludes M+2H and M+H+Na as being the compound's sole adduct on the basis of charge.

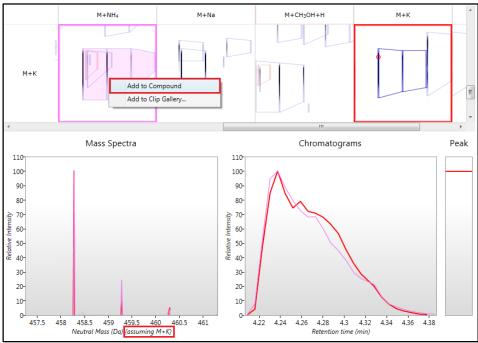


As an example to explore the posibility that **Compound 4.24_497.2361's** sole adduct is M+Na try hovering over the detected peaks for the expected adducts. In this case when you hover over M+H as the possible second adduct you can see that although the chromatograms elute at the same retention time the mass



spectra do not coincide. On this basis the second compound adduct cannot be M+H when M+Na is assumed to be the sole detected adduct.

Now try M+K as the assummed sole adduct of **Compound 4.24_497.2361**. This time when you hover over the cursor over the M+NH₄ adduct both the m/z on the mass spectra and RT on the chromatogram appear to coincide on the graphs with the corresponding location for the existing single adduct M+K.



To add the $M+NH_4$ adduct to the compound right click then add this second adduct to the compound. As you add the second adduct the table will update to indicate that the compound has 2 adducts, a **Neutral mass** is calculated (n appears after the compound in the table) and the matrix is replaced by a single row of adducts. This can be explored for the presence of additional adducts, in this case an additional 2 adducts can also be added (M+H and M+Na).

Exploring the expected location for the second adduct of a compound

After Peak picking there will be a list of compounds with a single adduct detected. For these compounds the review deconvolution montage will be displayed as a matrix where the process of deconvolution has been unable to assign a second adduct on the basis of any of the expected adducts displaying the correct m/z and retention time at the expected locations for any of the selected adduct ions.



Waters THE SCIENCE OF WHAT'S POSSIBLE." **Note**: As you hover the cursor over any of the expected locations for the second adduct the Mass spec and Chromatogram for the second adduct are displayed in the bottom panels. If both the m/z and RT appear to coincide (in this example the 'double headed' arrows indicate that neither coincide) with the corresponding location for the existing single adduct then Add this second adduct to the compound by right clicking on it and selecting **Add to compound**.

By adding the second adduct the montage and table will update to reflect the addition of the adduct to the compound and a Neutral mass will be assigned based on the 2 adducts. The matrix collapses to the single row displaying the locations of the two detected adducts.

Using 'Tagging' to filter data for identification

In order to identify the compounds you can focus on data that is showing significant differences between one or more groups by first creating tags for particular 'subsets' of your data. Then filter the displayed data using the tags to provide a targeted list of compounds to identify.

Before creating new tags you can remove the automatically generated 'Edited' you created in the previous section. Right click on the table and select 'Edit tags' click **Delete tag** and then **OK**.

As an example of the use of tags we will set out to identify the compounds that show a significant (Anova p-value < 0.05), 2 fold or greater increase in abundance for one of the conditions (A_LD, B_HD or C_Norm).

Edit Tags

To create a tag for all compounds displaying an Anova p value <0.05, right click on the Compounds table and select Quick tags then select **Anova p-value...**

Wo filter a	pplied			Create
Compound	Accepted ID	Tag 💌	Adducts	Addu
O 4.00_384.1998	n		M+H, M+NH4, M+Na, M+K	4 ^
O 4.33_486.2313	n		M+H, M+Na, M+K	3
o 5.00_440.1	No tags to assign		M+NH4, M+Na, M+K	3
0.47_459.7		_	M+H, M+Na, M+K	3
8.96_440.3	New tag		M+NH4, M+Na, M+K	3
o 9.57_582.4	Quick Tags	•	Anova p-value	3
0.46_195.8 🚰	Edit tags		Max fold change	3
0.46_700.6	Add to Clip Gallery		Minimum CV	3
0.55_331.0887	n	_	Not identified	3
0.46_405.7886r	n		Not fragmented	3
o 9.02_574.0949	n		Separated by drift time	3
0 8.96_396.2877r	n		Identified and separated by drift time	3
0.47_353.7767	n		M+H-H ₂ O, M+H, M+K	3

🛛 New Quick Tag
Where a compound has: Anova p-value: < 0.05
Apply the following tag:
Anova p-value ≤ 0.05
Create tag Cancel

Accept the default value (≤ 0.05) and the offered name.

On pressing **Create tag** a tag appears in the table against all the compounds with an Anova p-value ≤ 0.05

When the second	ilter applie	d			Create
Compound		Accepted ID	Tag 💌	Adducts	Addu
0 4.00_384	.1998n			M+H, M+NH ₄ , M+Na, M+K	4
0 4.33_486	.2313n			M+H, M+Na, M+K	3
o 5.00 440	.1694n		_	M+NH4, M+Na, M+K	3
0.4: 单	Anova p-	value ≤ 0.05	1	M+H, M+Na, M+K	3
0 8.96	New tag		•	M+NH4, M+Na, M+K	3
o 9.5	Quick Tag	js ►	Ano	va p-value	3
0.4	Edit tags		Max	fold change	3
0.46	Add to Cl	ip Gallery	Min	imum CV	3
0.55_551	.088/n		Not	identified	3
0.46_405	.7886n		Not	fragmented	3
0 9.02_574	.0949n		Sepa	arated by drift time	3
0 8.96_396	.2877n		Iden	tified and separated by drift time	3
0.47_353	.7767n			M+H-H2O, M+H, M+K	3

🔲 New Quick Tag 📃
Where a compound has: Max fold change: ≥ ▼ 2
Apply the following tag:
Max fold change ≥ 2
Create tag Cancel

To add a second 'Quick Tag' for those compounds with a Fold difference of 2 or greater, right click on the table to open the 'Tag' menu. Select Quick Tags and then Max fold change and accept the default value (\geq 2).

You will be offered a tag named **Max fold change \geq 2**.

On pressing **Create tag** a second tag appears in the table against all the compounds with a Max fold change \geq 2.

Now to get the table to display **only** those compounds which satisfy the required criteria, i.e. those compounds that display a significant 2 fold or greater increase in Mean abundance, apply a filter.

Wo filter ap	plied		Create	
Compound	Accepted ID	Tag 💌	Adducts	
O 4.93_426.1888n			M+NH4, M+Na, M+K	*
O 5.15_312.2079n		-	M+H-H ₂ O, M+H, M+NH ₄	
0 4.77_442.1479n		-	M+H-2H ₂ O, M+H-H ₂ O, M+H	
0 4.73_692.3840n			M+H, M+NH4, M+K	
○ 5.87_344.1838n		-	M+H-H ₂ O, M+Na, M+K	
○ 6.22_310.0805n		۵	M+H, M+Na, M+K	
○ 6.67_342.1801n			M+H, M+Na, M+K	
○ 7.36_470.2521n		-	M+NH4, M+Na, M+K	
0 7.64_484.2682n			M+NH4, M+Na, M+K	
○ 8.61_668.3078n			M+H-H ₂ O, M+Na, M+K	
0 8.66_640.1120n		-	M+H, M+NH4, M+Na	
0 8.96_396.2877n		-	M+H, M+NH4, M+Na	*

Create..

No filter applied

To set up a filter click **Create** to open the Filter dialog and drag the new tags on to the **Show** compounds with all of these tags.

Q Filter the compounds	
Create a filter Show or hide compounds based on a selection or to create the filter. For more guidance, please s	of their tags. Move tags to the appropriate boxes see the <u>online reference</u> .
Available tags: Anova p-value ≤ 0.05 (1258 compounds) Max fold change ≥ 2 (450 compounds) Clear the filter	Show compounds that have all of these tags: Show compounds that have at least one of these tags: Hide compounds that have any of these tags: OK Cancel

On clicking **OK** the table now displays only those compounds with the 2 tags. This example is ordered by number of adducts (adduct count)

Tag filter app compounds n			Edit
Compound	Accepted ID	Tag 💌	Adducts
• 7.11_308.1069n		4	M+H-H ₂ O, M+H, M+Na, M+K
○ 6.50_270.1040n		4	M+H, M+Na, M+K
O 6.22_308.0856n		4	M+H, M+Na, M+K
0 8.01_322.1212n		4	M+H, M+Na, M+K
0 8.20_322.2747n		4	M+H, M+Na, M+K
O 6.22_310.0805n		4	M+H, M+Na, M+K
0 8.21_322.1218n		4	M+H, M+Na, M+K
○ 7.13_308.1991n		4	M+H, M+Na, M+K
○ 7.12_366.9904n		4	M+NH ₄ , M+ACN+H
O 8.02_322.2790n		4	M+Na, M+K
O 2.90_213.0336n		4	M+Na, M+K

Note: the Tag filter panel has changed, informing you that a filter is currently applied.

With the filter still active click Section Complete to move to the Identify Compounds stage.

Stage 8: Identify Compounds

The user guide now describes how to identify compounds from **all or a subset** of compounds.

Note: that the filter you applied at the previous stage is currently active.

To display all the compounds in the table click **Edit** and then click on **Clear the filter** and then **OK**.

To Find **Compound 3.78_412.0375n**, which has a neutral mass of 412.0375, either scroll to the right in the table or expand the table by dragging the icon to the right of the table. Then order the table on descending number of adducts (Adduct count).

Ť								Crea	ate
Compound	Accepted ID	Tag 💌	Identifications	Fragmented?	Neutral mass	m/z	Drift time	Adduct count	
0 4.45_600.2999	n		0	Yes	600.2999	6	6.30		_
• 7.11_308.1069	n		0	Yes	308.1069	3 The m/	z of the m	ost abundant compound	
0 4.20_442.2051	n		0	Yes	442.2051			this, each ion's abundar sum of un-normalized	се
0.41_217.9769	n		0	Yes	217.9769			hat ion across all sample	es.
• 3.78_412.0375		-		Yes	412.0375	413.0448	3.73	4	_
0 4.40_586.3214	n		0	Yes	586.3214	604.3553	5.56	4	
0 4.66_648.3573	n		0	Yes	648.3573	666.3911	6.21	4	
0 6.67_447.2996	n		0	Yes	447.2996	448.3068	4.81	4	
0 4.05_454.2413	n		0	Yes	454.2413	455.2486	4.05	4	
0 3.82_245.1627	n		0	Yes	245.1627	287.1910	2.86	3	
0 3.53_296.1478	n		0	Yes	296.1478	335.1100	2.97	3	
o 4.21_413.9765	n		0	Yes	413.9765	432.0103	3.89	3	-

Tag filter applied

pted ID

compounds may be hidden

Search for identifications

Tag

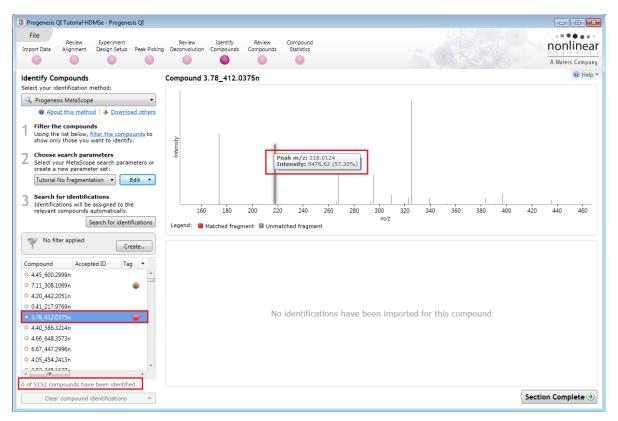
Create...

Edit.

gend:

⇔

At this stage the view displays the pattern of fragmentation for the selected compound



Note: the m/z displayed in the table is for the most abundant compound ion (in this case the M+H adduct). Currently there are 5152 compounds of which none have been identified.



Identification of compounds using the search method Progenesis MetaScope

This identification method is designed to support identifications from a number of different databases.

Depending on the available information in the database and the type of search being performed you can create and save re-usable **search parameter sets**.

For example: searching a database using compound values for Mass and Retention Time.

QI Progenesis QI Tutorial HDMSe - Progenesis QI	
File	Edit Search Parameters
Review Experiment Review Ider Import Data Alignment Design Setup Peak Picking Deconvolution Comp	etaScope search parameters
	fine a set of MetaScope parameters that can be saved for later A Waters Company
	use. Learn more in the <u>online reference</u> .
Identify Compounds Compound 3.7 Select your identification method: Na	me:
	utorial No Fragmentation
Progenesis Metascope	Compound database
	C:\Program Files (x86)\Nonlinear Dynamics\Prog
Filter the compounds	
Using the list below, filter the compounds to show only those you want to identify.	Data format: Auto-detect
	earch parameters
Select your MetaScope search parameters or create a new parameter set;	Precursor tolerance: 12 ppm
Tutorial No Fragmentation Edit	Retention time within: 0.1
Edit	
3 Search for identifications Identifications will be assigned to th	400 420 440 460
	Additional compound properties source
Search for ic Create a copy	Read additional compound properties from this file
No Elhan and Ead	C:\Users\andy.borthwick\Documents\Customer [
Vo filter applied	
-	ragment search method Do not use fragmentation data
	Perform theoretical fragmentation
o 3.78 437.5062m/z	Fragment tolerance: 12 ppm
	Perform fragment database search
0 2 79 420 5112-	<no database="" selected=""> Browse</no>
• 3.78_412.0375n 😑	
○ 3.78_411.0488m/z	Fragment tolerance: 12 ppm v
0 3.78_400.0568n	
 0 of 5152 compounds have been identified. 	Save search parameters Cancel
	Caption Convoluto (
Clear compound identifications -	Section Complete 🦻

First select the identification method from the top left of the screen. In this example for the Tutorial data use Progenesis MetaScope, a flexible search engine which is designed to work with databases where you can set thresholds for Mass, Retention time, Collisional Cross Section and Theoretical Fragmentation depending on the format and content of the database.

Set the Precursor tolerance to 12ppm, in this case you can use the **Tutorial No Fragmentation** search parameters as shown above.

Browse to locate the database file.

Location of example Structure Data Format (SDF) files after install:

C:\Program Files (x86)\Nonlinear Dynamics\Progenesis QI\Plugins\MetaScopeSearch\SDF Databases\

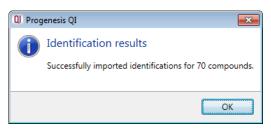
Select: Tutorial Fragmentation.sdf (if not already selected)

Progenesis QI User Guide

There are a number of Data Formats available including **Auto-detect**, which we will use here.

Save the Search Parameters and click Search for identifications.

A dialog will tell you the number of compounds that you have imported identifications for.



To focus only on the identified compounds, hide all the unidentified compounds. To do this you must first tag all the unidentified compounds. Right click on the table and select the Quick Tag for **Not identified.**

Then create a filter that hides these compounds by selecting Create and dragging the **Not identified** tag on to the **Hide** panel.

No filter ap	plied	e
Compound	Accepted ID Tag	-
 4.15_428.2277- 4.68_678.4 4.60_634.3 2.76_219.1 7.13_276.1 	Anova p-value ≤ 0.05 Max fold change ≥ 2 Not identified New taq	No identificati
0 0.77_520.1	Quick Tags 🔹 🕨	Anova p-value
 ○ 8.54_426.2 ○ 8.66_640.1 	Edit tags Add to Clip Gallery	Max fold change Minimum CV
○ 4.53_590.3537n	•	Not identified
○ 8.54_470.3255n	•	Not fragmented
< <u>III</u>		Separated by drift time
70 of 5152 compo	unds have been identified.	Identified and separated by drift time
Clear co	mpound identifications	•

Click **OK** to apply the filter.

Having imported the search results you can see there are a number of possible identifications for the identified compounds.

-	No filter applied	
Υ.	no mer oppned	Create

Possible identifications for the current compound are ranked on an overall **Score** based on, **Mass error**, **Retention Time error** (if available), **Isotope Similarity** (calculated from the comparison of the measured isotope distribution for the compound vs the theoretical based on the compound formula, **Fragmentation Score** and the compound's **Collisional Cross-Section** (CCS) if available.

Create a filter Show or hide compounds based on a selection poxes to create the filter. For more guidance,	
Available tags: Anova p-value ≤ 0.05 (1258 compounds) Max fold change ≥ 2 (450 compounds)	Show compounds that have all of these tags: Show compounds that have at least one of these tags:
	Hide compounds that have any of these tags: Not identified (5082 compounds)

Find **Compound 2.91_151.0638n.** The solid grey icon to the left of the **Compound** column indicates possible identifications have been made for this compound. With the available Retention time data for Paracetamol this would indicate that Paracetamol is a stronger match.

Compounds may be hidden	Edit		Compound ID	Description	Adducts	Formula	Retention time	Score	Fragmentation score	Mass error (ppm)	
Compound Accepted ID	Tag	\star	46506142	Paracetamol	M+H-H	C ₈ H ₉ NO ₂	2.90	51.3	0	3.46	
5.18_151.0638n	• •	☆	HMDB02210	2-Phenylglycine	M+H-H	$C_8H_9NO_2$		38.5	0	3.46	
5.18 179.0949n	<u> </u>	☆	HMDB12219	Dopamine quinone	M+H-H	C ₈ H ₉ NO ₂		38.5	0	3.46	HN
3.63_179.0946n		\Rightarrow	HMDB29703	Methyl 2-aminobenzoate	M+H-H	C ₈ H ₉ NO ₂		38.5	0	3.46	
2.96_314.1436n											
• 2.91_151.0639n	4										
4.29_162.0684n											
4.68_179.0954n											ľ
4.42_136.0758m/z											ОН
	*										OH
· · · · · · · · · · · · · · · · · · ·	•										

Auto-detect 🔹
Auto-detect
PubChem Compound
PubChem Substance
Lipid Maps
HMDB
DrugBank
KEGG
RCSB PDB
ChemDB
ChemBank
Mcard
CAS
ChEBI

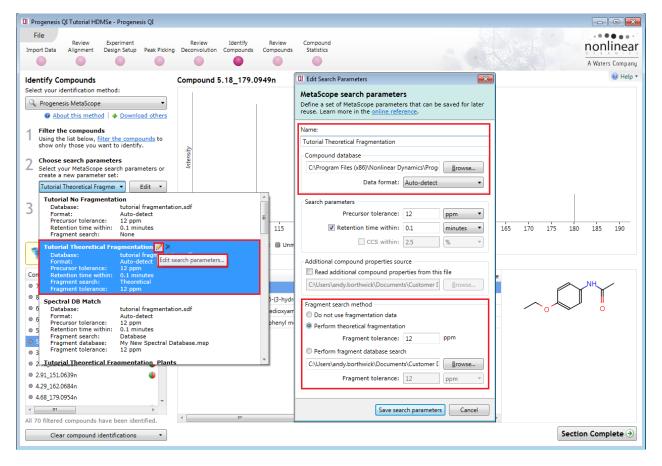
Note: when the Retention time data is **not** included as part of the search scoring (by unticking the option on the search parameters) then there is no difference in the scoring for the 4 identification candidates in the table to the right.

🐨 Tag filter appli	ed 📂		Pos	sible identific	ations: 4						
Compounds may	y be hidden 📃	Edit	\Rightarrow	Compound ID	Description	Adducts	Formula	Retention time	Score	Fragmentation score	Mass error (ppm)
Compound	Accepted ID	Tag	\star	46506142	Paracetamol	M+H-H	C ₈ H ₉ NO ₂	2.90	38.5	0	3.46
• 5.18 151.0638n		- (\Rightarrow	HMDB02210	2-Phenylglycine	M+H-H	C ₈ H ₉ NO ₂		38.5	0	3.46
● 5.18 179.0949n			\Rightarrow	HMDB12219	Dopamine quinone	M+H-H	C ₈ H ₉ NO ₂		38.5	0	3.46
 3.63_179.0946n 			\Rightarrow	HMDB29703	Methyl 2-aminobenzoate	M+H-H	C ₈ H ₉ NO ₂		38.5	0	3.46
© 2.96_314.1436n											
• 2.91_151.0639n		4									
4.29_162.0684n											
4.68_179.0954n											
4.42_136.0758m/z											
< III /		+ ·									
Il 70 filtered compour	nds have been id	dentified.	٩ [•

Incorporating Theoretical Fragmentation

To improve the confidence in the compound identification you can choose to perform theoretical fragmentation of a candidate list of compounds and then match the resulting 'in silico' fragmentation against the measured/observed fragments for a compound. The candidate molecules are selected from a compound database based on the exact mass (within a specified error range given in (ppm).

The compound database is in SDF format providing the structural information for each candidate compound. Using this list of candidates the fragmentation algorithm generates all possible fragments for a candidate compound in order to match the fragment mass with the measured peaks.



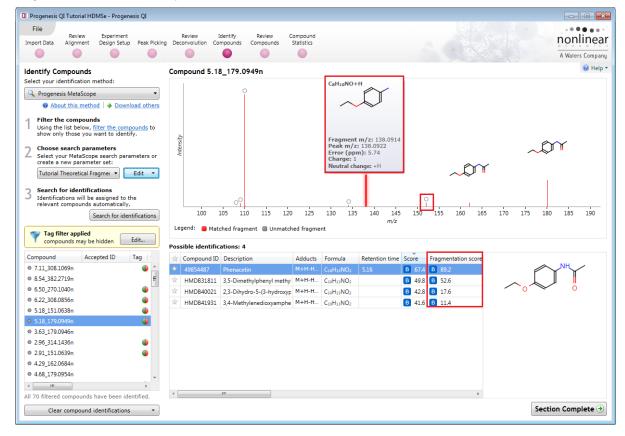
To perform theoretical fragmentation using the **tutorial fragmentation .sdf** as the source of the candidate compounds select the second Search Parameter Set (Tutorial Theoretical Fragmentation) from the drop down

Note: you can review/edit the parameter set, either by clicking on the 'pencil' icon in the drop down or using the **Edit** option to the right of the drop down.

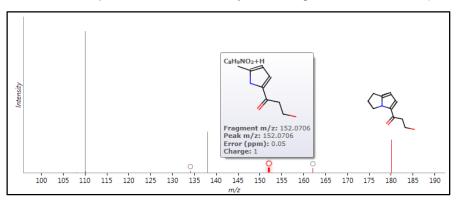
Ensure the **Perform theoretical fragmentation** from the Fragment search method is selected and then save search parameters.

To perform the Theoretical Fragmentation search click on Search for Identify Compounds identifications Select your identification method: Progenesis MetaScope About this method | + Download others As before search 70 results will be returned. Filter the compounds Using the list below, <u>filter the compou</u> show only those you want to identify. pounds to QI Progenesis QI X Choose search parameters Identification results Select your MetaScope search parameters or create a new parameter set: Successfully imported identifications for 70 compounds. Tutorial Theoretical Fragmenta 🔻 Edit 🔹 Search for identifications 3 Identifications will be assigned to the relevant OK compounds automatically. Search for identifications

Where fragments have been measured for a compound and a match is made with the theoretical fragments from each candidate compound a solid coloured line appears on the Fragmentation graph. This is accompanied with either an image of the fragment displayed (if there is room) or an open symbol at the top of the line which enables display of the fragment when the cursor is held over it (as shown below for **Compound 5.18_179.0949n**).



The fragmentation score for each possible identity appears in the table. When you click on one of the possible identifications with a lower fragmentation score the Fragmentation graph displays which fragments contribute to that score for example the **3**rd **ranked compound** Fragmentation matches (see below).



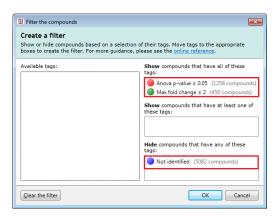
Note: a fragmentation score of 0 indicates either that **no** match was achieved between the theoretical fragments (of a candidate compound) and the measured fragments or in fact the compound has no fragmentation data. For example, in **Compound 2.58_169.0965m/z**:

QI Progenesis QI Tutorial HDMSe - Progenesis QI		- • •
File Import Data Alignment Design Setup Peak Picki	g Deconvolution Compounds Compounds Statistics	nonlinear A Waters Company
Identify Compounds Select your identification method:	Compound 2.58_169.0965m/z	Ø Help ▼
Progenesis MetaScope About this method Download others		
 Filter the compounds Using the list below, filter the compounds to show only those you want to identify. 		
2 Choose search parameters Select your MetaScope search parameters or create a new parameter set:	This compound has no fragmentation data	
Tutorial Theoretical Fragmer Edit Search for identifications		
Identifications will be assigned to the relevant compounds automatically.		
Search for identifications	Possible identifications: 4	
Tag filter applied	Compound ID Description Adducts Formula Retention time Score Fragmentation score	он
compounds may be hidden Edit	HMDB12219 Dopamine quinone M+NH4 CgH9NO2	
Compound Accepted ID Tag		H ₂ N
7.36_463.1712m/z	★ HMDB02210 2-Phenylglycine M+NH4 C∉H9NO2 A 37.1 A 0	\sim
 7.40_405.1712m/z 7.40_406.1727m/z 	☆ 46506142 Paracetamol M+NH₄ C ₈ H ₉ NO ₂ 2.90 A 37.1 A 0	
• 2.58 169.0965m/z		
2.61_221.1282m/z		
2.44_144.0803m/z		
< >		\checkmark
All 70 filtered compounds have been identified.	4 <u> </u>	
Clear compound identifications •		Section Complete

Note: where multiple searches have been performed the search yielding the highest overall score for each candidate is displayed. Also the search parameters giving rise to these scores are displayed as tooltips when the cursor is held over the Search Parameter indicator.

QI Progenesis QI Tutorial HDMSe - Progenesis QI		
File Review Experiment Import Data Alignment Design Setup Peak Picking	Review Identify Review Compound Deconvolution Compounds Compounds Statistics	nonlinear A Waters Company
Identify Compounds Select your identification method: Progenesis MetaScope	Compound 2.91_151.0639n	
relevant compounds automatically. Search for identifications	Possible identifications: 4	
Tag filter applied compounds may be hidden Edit Compound Accepted ID Tag 0 5.18,179.0949n • • 0 2.30,3179.0946n • • 0 2.90,314.1435n • • 0 4.29,162.0684n • • All 70 filtered compounds have been identified. Clear compound identifiedtions •	Image: marked bit with the second s	Score Fragmentation score 9 09.7 B 92 B 563 B 89 A 385 Method: Progenesis MetaScope Database: tutoial fragmentation.sdf Precursor tolerance: 12 ppm Retention time within: 0.1 minutes Theoretical fragment tolerance: 12 ppm , OH Section Complete \textcircled{P}

The table of compounds can be reduced to displaying only those compounds that have been identified, show a significant difference between the conditions, and are altered by at least two-fold, by applying a 'Tag Filter' based on the assigned 'Quick tags.



The table now displays only information for 20 identified compounds.

Q Progenesis QI Tutorial HDMSe - Progenesis QI		
File Review Experiment Import Data Alignment Design Setup Peak Picking	Review Identify Review Compound Decomolution Compounds Compounds Statistics	nonlinear A Waters Company
Identify Compounds	Compound 7.11_308.1069n	🛞 Help 🔻
Select your identification method:		
Progenesis MetaScope		
About this method Download others		
1 Filter the compounds Using the list below, filter the compounds to show only those you want to identify.	Internity	Ŷ.
2 Choose search parameters Select your MetaScope search parameters or create a new parameter set:		
Tutorial Theoretical Fragmer 🔻 Edit 👻	9	
3 Search for identifications Identifications will be assigned to the	130 140 150 160 170 180 190 200 210 220 230 240 250 2 m/z	60 270 280 290 300 310 320 330 340 350
relevant compounds automatically.	Legend: 🗧 Matched fragment 🔳 Unmatched fragment	
Search for identifications	Possible identifications: 1	
Tag filter applied	Compound ID Description Adducts Formula Retention time Score	Fragmentation score
compounds may be hidden		B 82.4
Compound Accepted ID Tag		
• 7.11_308.1069n		
• 6.22_308.0856n		
© 6.50_270.1040n		
© 5.18_151.0638n		
© 5.18_179.0949n		Ŭ T
• 2.91_151.0639n		
© 2.96_314.1436n		
< >	4	•
All 20 filtered compounds have been identified.		
Clear compound identifications -		Section Complete 🤿

Creating and using databases

The identification data can be stored and used to perform fragment database searches. This allows you to build up your own (local) databases of fragment information. These can then be used to perform future identifications based on fragment matches when using the same compound database. To create and subsequently export data to such a database you must indicate compounds to export by accepting their identity.

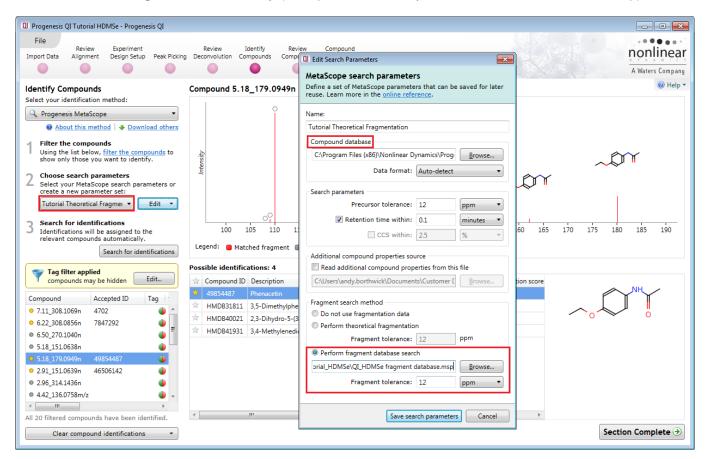
Q	Progenesis QI Tutorial HDMSe - Progenesis QI		
	File		
	Save	Review Identify Review Compound Deconvolution Compounds Compounds Statistics	nonlinear
	Close		A Waters Company
	Export compound measurements	Compound 5.18_179.0949n (Phenacetin)	
	Export compound identifications Export isotope information	0	_
Г	Export fragment database	QI Export fragment database	
F	Export additional compound properties Export to pathways tool	Select which of the accepted identifications for your filtered compound ions you want to export.	~~
	Import compound identifiers as tag	Compound Accepted ID Adducts	~~~~ "
	Export to EZinfo	S 7.11_308.1069n 4702 M+H, M+K, M+Na, M+H-H ₂ O	
	Open EZinfo project		
		✓ 5.18_179.0949n 49854487 M+H-H₂O, M+H	
	Export inclusion list	✓ 2.91_151.0639n 46506142 M+H-H₂O, M+H	
	Experiment properties	100 105 V 3.69_195.0897m/z 46511425 M+H	170 175 180 185 190
	Show Clip Gallery	Legend: Matched	
×	Exit	About fragment databases Export Cancel	
	compounds may be hidden Edit	Compound ID Description Adducts Formula Retention time Score Fragmentation score	_
	ompound Accepted ID Tag	★ 49854487 Phenacetin M+H-H C ₁₀ H ₁₃ NO ₂ 5.16 B 67.4 B 89.2	NH
	7.11_308.1069n 4702	☆ HMDB31811 3,5-Dimethylphenyl methyl M+H-H C ₁₀ H ₁₃ NO ₂ ■ 49.8 ■ 52.6	
	6.22 308.0856n 7847292	☆ HMDB40021 2,3-Dihydro-5-(3-hydroxyp M+H-H C10H13NO2 B 42.8 B 17.6	- 0 * 0
0	6.50_270.1040n	☆ HMDB41931 3,4-Methylenedioxyamphe M+H-H C ₁₀ H ₁₃ NO ₂ B 41.6 B 11.4	
0	5.18_151.0638n 🕒 🕌		
•	5.18_179.0949n 49854487 🌗		
	2.91_151.0639n 46506142 🌒		
	2.96_314.1436n		
e	4.42_136.0758m/z		
	20 filtered compounds have been identified.	<)	
	Clear compound identifications		Section Complete 🏵

Using the Filter set created for the 20 compounds in the previous section, accept the identity of a compound by clicking on the 'star' icon to the left of the Compound ID, it will turn yellow. Do this for a number of compounds displaying an acceptable fragmentation score.

Then select **Export fragment database...** from the file menu as shown above.



When you export these accepted identifications the file is saved as a Mass Spectrometry Profile (msp) file. i.e. **QI_HDMSe fragment database.msp** (example: in the folder you restored the Tutorial Data Set.zip).



You can add to this file as you gather additional information from other experiments.

Now as an example re-perform the Tutorial Theoretical Fragmentation but this time instead of selecting the **Perform theoretical fragmentation** option select the **Perform fragment database search** option. Then locate the .msp file you have just created.

Then save the Search parameters and perform the Fragment database search by clicking on **Search for identifications**.

Note: you must use the same Compound database that was used when the original msp file was generated. This is because the initial step of Progenesis QI using a fragment database is to find all possible identifications based on neutral mass (RT and CCS used if available). Then for each possible identification (candidate) Progenesis QI searches the fragment database for a record with a matching compound ID. It then compares the stored fragment data to the current experimental fragmentation data.

On performing the search you will get a 'mirror plot' where the upper half represents the measured/observed fragmentation and the lower half shows the currently stored fragmentation in the database. This gives rise to a **Fragmentation score** describing how good the match is between the observed fragmentation and the stored fragmentation in the database.

The fragmentation score is based on the number of fragments matched and their intensity. This excludes any contribution from the matching of the precursor.

The current search with the highest score is designated C in this example (Compound 5.18_179.0949n).

File Review Experiment Import Data Alignment Design Setup Peak Picking	Review Ide Deconvolution Com		Compound Statistics					nonlinea
		•						A Waters Compar
dentify Compounds	Compound 5.18	179.0949n (Phenac	etin)					🕜 Help
Select your identification method:			,					
Progenesis MetaScope								
About this method + Download others	nred							
Filter the compounds	Measured							
Using the list below, filter the compounds to								1
show only those you want to identify.								
Choose search parameters	v							
Select your MetaScope search parameters or create a new parameter set:	Database							
Tutorial Theoretical Fragmer 💌 🛛 Edit 💌	Dat							
Search for identifications Identifications will be assigned to the relevant compounds automatically	100 10	05 110 115 120	0 125 130	135 140 14 m/z		5 160 165	170 175	5 180 185 190
Jentifications will be assigned to the relevant compounds automatically.	Legend: 📒 Match	ned fragment 🔲 Unmatch		135 140 14 <i>m/z</i>		5 160 165	170 175	5 180 185 190
Identifications will be assigned to the relevant compounds automatically. Search for identifications	Legend: Match Possible identificat	hed fragment 🔲 Unmatch		m/z			170 175	5 180 185 190
Identifications will be assigned to the relevant compounds automatically. Search for identifications Tag filter applied compounds may be hidden Edit.	Legend: Match Possible identificat	hed fragment 🔲 Unmatch	hed fragment	m/z ula Retention time		mentation score	170 175	5 180 185 190
Identifications will be assigned to the relevant compounds automatically. Search for identifications Tag filter applied compounds may be hidden Edit. Compound Accepted ID Tag if	Legend: Match Possible identificat Compound ID A9854487	tions: 4 Description	Adducts Formu M+H-H C ₁₀ H1	m/z ula Retention time 1NO2 5.16	Score Frag	mentation score 100	170 175	5 180 185 190
Identifications will be assigned to the relevant compounds automatically. Search for identifications Tag filter applied compounds may be hidden Edit Compound Accepted ID Tag i 9.7.11_308.1069n 4702	Legend: Match Possible identificat Compound ID I A 49854487 C HMDB31811	hed fragment Unmatch tions: 4 Description Phenacetin	Adducts Formu M+H-H C ₁₀ H ₁₁ M+H-H C ₁₀ H ₁₂	m/z ula Retention time 1NO2 5.16 1NO2	Score Frag	mentation score 100 52.6	170 175	
Identifications will be assigned to the relevant compounds automatically. Search for identifications Tag filter applied compounds may be hidden Edit. Compound Accepted ID Tag 7.31_308.1069n 4702	Legend: Match Possible identificat	ed fragment Unmatch tions: 4 Description Phenacetin 3,5-Dimethylphenyl methyl	Adducts Formu M+H-H C ₁₀ H ₁₁ M+H-H C ₁₀ H ₁₂ M+H-H C ₁₀ H ₁₂	m/z ula Retention time 1NO2 5.16 1NO2 1NO2	 Score Frag C 69.5 C B 49.8 B 	mentation score 100 52.6 17.6	170 175	
Identifications will be assigned to the relevant compounds automatically. Search for identifications Tag filter applied compounds may be hidden Edit Compound Accepted ID Tag 7.11_308.1069n 4702 6.52_2308.0856n 7847292 6.550_270.1040n	Legend: Match Possible identificat	tions: 4 Description Phenacetin 3,5-Dimethylphenyl methyl 2,3-Dihydro-5-(3-hydroxyp	Adducts Formu M+H-H C ₁₀ H ₁₁ M+H-H C ₁₀ H ₁₂ M+H-H C ₁₀ H ₁₂	m/z ula Retention time 1NO2 5.16 1NO2 1NO2	Score Frag C 69.5 C B 49.8 B B 42.8 B	mentation score 100 52.6 17.6	170 175	
Identifications will be assigned to the relevant compounds automatically. Search for identifications Y Tag filter applied compounds may be hidden Compound Edit. Compound Accepted ID 7.11_308.1069n 4702 6.22_308.0856n 7847292 6.50_270.1040n ● 5.18_151.0638n ●	Legend: Match Possible identificat	tions: 4 Description Phenacetin 3,5-Dimethylphenyl methyl 2,3-Dihydro-5-(3-hydroxyp	Adducts Formu M+H-H C ₁₀ H ₁₁ M+H-H C ₁₀ H ₁₂ M+H-H C ₁₀ H ₁₂	m/z ula Retention time 1NO2 5.16 1NO2 1NO2	Score Frag C 69.5 C B 49.8 B B 42.8 B	mentation score 100 52.6 17.6	170 175	
Identifications will be assigned to the relevant compounds automatically. Search for identifications Tag filter applied compounds may be hidden Edit. Compound Accepted ID Tag Accepted I	Legend: Match Possible identificat	tions: 4 Description Phenacetin 3,5-Dimethylphenyl methyl 2,3-Dihydro-5-(3-hydroxyp	Adducts Formu M+H-H C ₁₀ H ₁₁ M+H-H C ₁₀ H ₁₂ M+H-H C ₁₀ H ₁₂	m/z ula Retention time 1NO2 5.16 1NO2 1NO2	Score Frag C 69.5 C B 49.8 B B 42.8 B	mentation score 100 52.6 17.6	170 175	
Identifications will be assigned to the relevant compounds automatically. Search for identifications Tag filter applied compounds may be hidden Edit. Compound Accepted ID Tag Accepted I	Legend: Match Possible identificat	tions: 4 Description Phenacetin 3,5-Dimethylphenyl methyl 2,3-Dihydro-5-(3-hydroxyp	Adducts Formu M+H-H C ₁₀ H ₁₁ M+H-H C ₁₀ H ₁₂ M+H-H C ₁₀ H ₁₂	m/z ula Retention time 1NO2 5.16 1NO2 1NO2	Score Frag C 69.5 C B 49.8 B B 42.8 B	mentation score 100 52.6 17.6	170 175	
I dentifications will be assigned to the relevant compounds automatically. Search for identifications Tag filter applied Edit. Compound Accepted ID Tag Compound Accepted ID	Legend: Match Possible identificat	tions: 4 Description Phenacetin 3,5-Dimethylphenyl methyl 2,3-Dihydro-5-(3-hydroxyp	Adducts Formu M+H-H C ₁₀ H ₁₁ M+H-H C ₁₀ H ₁₂ M+H-H C ₁₀ H ₁₂	m/z ula Retention time 1NO2 5.16 1NO2 1NO2	Score Frag C 69.5 C B 49.8 B B 42.8 B	mentation score 100 52.6 17.6	170 175	
Identifications will be assigned to the relevant compounds automatically. Search for identifications Tag filter applied Edit. Compound Accepted ID Tag Attached Tag Att	Legend: Match Possible identificat	tions: 4 Description Phenacetin 3,5-Dimethylphenyl methyl 2,3-Dihydro-5-(3-hydroxyp	Adducts Formu M+H-H C ₁₀ H ₁₁ M+H-H C ₁₀ H ₁₂ M+H-H C ₁₀ H ₁₂	m/z ula Retention time 1NO2 5.16 1NO2 1NO2	Score Frag C 69.5 C B 49.8 B B 42.8 B	mentation score 100 52.6 17.6	170 175	

You can also manage and search against additional compound properties. The advantage of this is that you can create a database of compound data that retains information on an identified compound for m/z, retention time. Export and save this **Additional Compound properties** file.

Q Progenesis QI Tutorial HDMSe - Progenesis QI		- • •
File		
Save	Review Identify Review Compound no	onlinear
Close		aters Company
Export compound measurements		Help
	Compound 5.18_179.0949n (Phenacetin)	W Help *
Export compound identifications		
Export isotope information	2 Q Export additional compound properties	
Export fragment database	QI Export additional compound properties Select which filtered compound ions you want to export.	
Export additional compound properties		
Export to pathways tool	Compound Accepted ID Retention Time (mins) CCS Adduct	
Import compound identifiers as tag	✓ 7.11_308.1069n 4702 7.11 ✓ 6.22_308.0856n 7847292 6.22	
Export to EZinfo		
Open EZinfo project	Image: State State State Image: State State State State Image: State State State State State Image: State State<	
French Industry Ed.	☑ 3.69 195.0897m/z 46511425 3.69	
Export inclusion list		35 190
Experiment properties Show Clip Gallery		D TAO
show Cip Gallery	Legend: About additional compound properties files Export Cancel	
× Exit	Possible identifications: 4	
compounds may be hidden Edit	Compound ID Description Adducts Formula Retention time Score Fragmentation score	
Compound Accepted ID Tag	🐓 49854487 Phenacetin M+H-H C10H13NO2 5.16 € 69.5 € 100	\checkmark
	★ HMDB31811 3,5-Dimethylphenyl methyl M+H-H C10H13NO2 B 49.8 B 52.6	
6.22 308.0856n 7847292	★ HMDB40021 2,3-Dihydro-5-(3-hydroxyp M+H-H C ₁₀ H ₁₃ NO ₂ B 42.8 B 17.6	0
• 6.50_270.1040n	★ HMDB41931 3,4-Methylenedioxyamphe M+H-H C10H13NO2 B 41.6 B 11.4	
• 5.18_151.0638n		
• 5.18_179.0949n 49854487 🌐		
• 2.91_151.0639n 46506142		
© 2.96_314.1436n		
• 4.42_136.0758m/z		
	< III >	
All 20 filtered compounds have been identified.		
Clear compound identifications -	Section Co	mplete 🔿

Note: if the data format contains Drift time data and it has been calibrated then the value for a compounds Collisional Cross Section (CCS) may also be stored as an additional compound property and used in the process of compound identification.

Deleting Compound Identifications

Before using the **additional compounds properties** file, you can explore how to clear compound identifications. If more than one Search Configuration has been used then you can remove identifications based on the Configuration. To do this click on **Clear compound identifications** and select the configuration.

QI Progenesis QI Tutorial HDMSe - Progenesis QI		
File Review Experiment Import Data Alignment Design Setup Peak Picking	Review Identify Review Compound Deconvolution Compounds Statistics	nonlinear
		A Waters Company
Identify Compounds Select your identification method:	Compound 5.18_179.0949n (Phenacetin)	🛞 Help 🔻
Progenesis MetaScope	a a a a a a a a a a a a a a a a a a a	
About this method + Download others	Messured	
Filter the compounds Using the list below, filter the compounds to show only those you want to identify.	W	
2 Choose search parameters Select your MetaScope search parameters or create a new parameter set: Tutorial Theoretical Fragmer Edit	Database	I
3 Search for identifications Identifications will be assigned to the relevant compounds automatically. Search for identifications	100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 Legend: Matched fragment Unmatched fragment	175 180 185 190
Tag filter applied	Possible identifications: 4	
compounds may be hidden Edit	Compound ID Description Adducts Formula Retention time Score Fragmentation score	A NH /
Compound Accepted ID Tag	Image: Weight and the state of th	
• 7.11_308.1069n 4702	★ HM0851011 3,5*0imetry preny metry M+H-H C ₁₀ H ₁₃ NO ₂ Ø 45.8 Ø 52.8 ★ HM0840021 2,3-0ihydro-5-(3-hydroxyp M+H-H C ₁₀ H ₁₃ NO ₂ B 42.8 B 17.6	
6.22_308.0856n 7847292 6.50 270.1040n	☆ HMDB41931 3,4-Methylenedioxyamphe M+H-H C ₁₀ H ₁₃ NO ₂ B 41.6 B 11.4	
• 5.18 151.0638n		
0 5 18 179 0949n 49854487 4		
Clear identifications from search A (Progenesis M)	etaScope)	
Clear identifications from search B (Progenesis M		
Clear identifications from search C (Progenesis M	tascope)	
× Clear all identifications	· · · · · · · · · · · · · · · · · · ·	
Clear compound identifications 🔹		Section Complete 🤿

When deleting identifications by Search configuration (in this example B) you will be warned which ones will be cleared.



Following deleting the identifications based on Search configuration B there will only be one possible remaining identification for **Compound 5.18_179.0949n**

compounds may be hidde	n Edit	Compound ID	Description	Adducts	Formula	Retention time		Fragmentation score	
Compound Accepted	ID Tag	* 49854487	Phenacetin	M+H-H	C ₁₀ H ₁₃ NO ₂	5.16	C 69.5	C 100	
▶ 7.11_308.1069n									
6.22_308.0856n	•								0 1 0
0 6.50_270.1040n	• 1								
5.18_151.0638n									
• 5.18_179.0949n	٩								
© 2.91 151.0639n	4		1						
Clear identifications from s	earch A (Progenesis Met	(Scope)							
Clear identifications from s	earch C (Progenesis Met	Scope)							
Clear all identifications								,	

Now clear all the compound identifications and re-perform the **Tutorial No Fragmentation** search as described on page 39.

To make use of the **Additional compound properties file**, you created on page 46, either edit, copy or make a new set of search parameters using this file as the source. In the example below we have edited the existing search parameters for the **Tutorial no Fragmentation** search parameters.

Q Progenesis QI Tutorial HDMSe - Progenesis QI			
File Review Experiment Import Data Alignment Design Setup Peak Picking	Review Deconvolutio	Letit Search Parameters MetaScope search parameters Define a set of MetaScope parameters that can be saved for later reuze. Learn more in the online reference.	A Waters Company
Identify Compounds	Compound		🕜 Help 🔻
Select your identification method:		Name:	
🔍 Progenesis MetaScope 🔹		Tutorial No Fragmentation	
About this method Download others		Compound database	
Filter the compounds		C:\Program Files (x86)\Nonlinear Dynamics\Prog Browse	
Using the list below, <u>filter the compounds</u> to show only those you want to identify.	Intensity	Data format: Auto-detect 🔹	
2 Choose search parameters Select your MetaScope search parameters or	Inte	Search parameters	
create a new parameter set:		Precursor tolerance: 12 ppm -	
Tutorial No Fragmentation 💌 Edit 👻		Retention time within: 0.1 minutes •	
3 Search for identifications Identifications will be assigned to the	10	CCS within: 2.5 %	45 150 155 160 165 170 175 180 185 190
relevant compounds automatically.	Legend:	Additional compound properties source	
Search for identifications	Legend:	Read additional compound properties from this file	
Tag filter applied	Possible ide	QI_HDMSe Additional Compound Properties.csv Browse	
compounds may be hidden Edit	☆ Compou	Fragment search method	e Score Fragmentation score
Compound Accepted ID Tag	* 498544	Do not use fragmentation data	49.5 0
● 7.11_308.1069n 🕘 📥	HMDB4	Perform theoretical fragmentation	39.3 0
• 6.22_308.0856n	HMDB4	Fragment tolerance: 12 ppm	39.3 0
© 6.50_270.1040n	🖄 HMDB	Perform fragment database search	39.3 0
• 5.18_151.0638n		<no database="" selected=""> Browse</no>	
• 5.18_179.0949n			
• 2.91_151.0639n		Fragment tolerance: 12 ppm *	
• 2.96_314.1436n			
• 4.42_136.0758m/z		Save search parameters Cancel	
All 20 filtered compounds have been identified.	4		•
Clear compound identifications •			Section Complete $ e e e e e e e e e e e e e $

When you re-perform the search a higher overall score is achieved using the additional scored compound data.

(
QI Progenesis QI Tutorial HDMSe - Progenesis QI		
File		
Review Experiment Import Data Alignment Design Setup Peak Picking	Review Identify Review Compound Deconvolution Compounds Compounds Statistics	nonlinear
		A Waters Company
		100
Identify Compounds	Compound 5.18_179.0949n	🔞 Help 🔻
Select your identification method:		
🔍 Progenesis MetaScope 🔹		
About this method Download others		
4 Filter the compounds		
Using the list below, filter the compounds to	4	
show only those you want to identify.	Internsity	
7 Choose search parameters	5	
Select your MetaScope search parameters or create a new parameter set:		
Tutorial No Fragmentation Edit		
2 Search for identifications	100 105 110 115 120 125 130 135 140 145 150 155 160 165 170	175 180 185 190
Identifications will be assigned to the relevant compounds automatically.	100 103 110 113 120 123 130 135 140 143 130 135 160 163 170 m/z	1/2 180 183 190
Search for identifications	Legend: 📒 Matched fragment 🔲 Unmatched fragment	
Tag filter applied	Possible identifications: 4	
compounds may be hidden Edit	☆ Compound ID Description Adducts Formula Retention time Score Fragmentation score	A NH A
Compound Accepted ID Tag	★ 49854487 Phenacetin M+H-H C ₁₀ H ₁₃ NO ₂ 5.18 B 59.2 B 0	
● 7.11_308.1069n	Image: Microsoft Micros	
6.22_308.0856n	Image: Main and Ain and	0 - 0
● 6.50_270.1040n	☆ HMDB31811 3,5-Dimethylphenyl methyl M+H-H C10H13NO2 ▲ 39.3 ▲ 0	
• 5.18_151.0638n		
• 5.18_179.0949n		
© 2.91_151.0639n		
• 2.96_314.1436n		
• 4.42_136.0758m/z		
< >		
All 20 filtered compounds have been identified.	< >	
Clear compound identifications -		Section Complete 🏵
		· -]

Note: the saved **Additional Compound Properties** file is the source of the 'in house' saved compound properties (Retention time and CCS) while the Compounds database file provides the neutral mass and structure details.

Each matching component (where available) contributes 20% to the overall score: Mass error, Retention time, Isotope similarity, Fragmentation score and Collisional Cross-Section (CCS).

i.e. if only mass error and isotope similarity are available then the maximum score will be out of 40.

Additional Identification Methods

In addition to Metascope and Metlin, Progenesis QI v2.2 has identification methods for Elemental Composition, LipidBlast, ChemSpider and NIST. With the exception of NIST, these methods are installed by default and are available at the Identify Compounds stage from the identification method drop down.

QI Edit Search Parameters

×

🕜 Help ୟ

190

MetaScope search parameters Define a set of MetaScope parameters that can be saved for later reuse. Learn more in the <u>online reference</u>. As an example of using Elemental Composition: first Clear all Name compound identifications that you carried out in the previous section, Tutorial Theoretical Fragmentation and leave the current Tag Filter applied. Compound database C:\Program Files (x86)\Nonlinear Dynamics\Prog Data format: Auto-detect Search parameters Precursor tolerance: 12 ppm Then with MetaScope still selected as the current identification method Retention time within: 0.1 minutes 🔹 return the Tutorial Theoretical Fragmentation search parameters to CCS within: 2.5 % the default settings as shown to the right by ticking Perform Additional compound properties source theoretical fragmentation. Save the search parameters. Read additional compound properties from this file C:\Users\andy.borthwick\Documents\Customer [Browse... Fragment search method Do not use fragmentation data Perform theoretical fragmentation Finally re-perform the search for identifications using the Tutorial Fragment tolerance: ppm Theoretical Fragmentation parameter set. Perform fragment database search C:\Users\andy.borthwick\Documents\Customer [Browse... Fragment tolerance: 12 ppm Save search parameters Cancel I Progenesis QI Tutorial HDMSe - Progenesis QI - • × File Review Experiment Review Identify Review Compound nonlinear Alignment Import Data Design Setup Peak Picking Deconvolution Compou nds Compounds Statistics A Waters Company Identify Compounds Compound 5.18_179.0949n Select your identification method: 🔍 Progenesis MetaScope ChemSpider Version: 1.0.5770.47720 🔍 LipidBlast Version: 1.0.5766.33115 Progenesis MetaScope Version: 1.0.5770.47506 🔍 METLIN batch metabolite search NIST MS/MS Library Version: 1.0.5673.40082 Toentineations will be assigned to t relevant compounds automatically. 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 Search for identifications Legend: 📕 Matched fragment 🔲 Unmatched fragment Tag filter applied Possible identifications: 4 compounds may be hidden Edit... Retention time Score Compound ID Description Adducts Formula Fragmentation score Compound Accepted ID Tag M+H-H.. • 7.11_308.1069n 4 HMDB31811 3,5-Dimethylphenyl methyl M+H-H... C10H13NO2 49.8 52.6

4.42 136.0758m/z ٩ 4.62_152.0702m/z ٩ Þ All 20 filtered compounds have been identified. Clear compound identifications -

HMDB40021 2,3-Dihydro-5-(3-hydroxyp M+H-H... C10H13NO2

HMDB41931 3,4-Methylenedioxyamphe M+H-H... C10H13NO2

42.8

41.6

17.6

11.4

Then select Elemental composition as the Identification methods.

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4

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\$

6.22_308.0856n

6.50 270.1040n

© 5.18 151.0638n

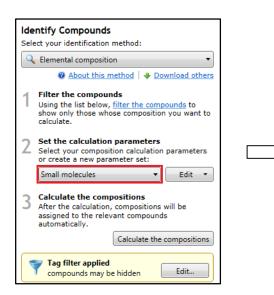
5.18 179.0949n

• 2.91_151.0639n

• 2.96_314.1436n

Section Complete 🤿

There are 3 default parameter sets: Small molecules, Lipids and CHNO each containing a list of Elements to be considered and thresholds for **Precursor tolerance (ppm/Da)** and **Isotope similarity (%)**.



	tify Compounds t your identification met	hod:	
Q	Elemental composition		•
	About this method	od 🖊 Downloa	ad others
	Filter the compounds Using the list below, <u>filte</u> show only those whose o calculate.		
_	Set the calculation par Select your composition or create a new paramet	calculation para	meters
	Small molecules	• E	dit 🔹
3	Small molecules Precursor tolerance: Isotope similarity: Elements:	3 ppm	e
	Lipids Precursor tolerance: Isotope similarity: Elements:		ositions
Con 6	CHNO Precursor tolerance: Isotope similarity: Elements:	3 ppm 95.00% C, H, N, O	·

Select the **Small molecules** parameter set and click **Calculate the compositions**.

Click OK to import the 6 identifications.

QI Prog	enesis QI
i	Identification results Successfully imported identifications for 6 compounds.
	ОК

The **Possible identifications** table for the current compound updates to include any compositions that meet the current calculation parameters.

File											
Review Experiment	Review g Deconvolution Co		view Compound bounds Statistics							nonl	inear
	•									A Waters	Company
dentify Compounds	Compound 5.1	18 179.0949r									🕜 Help 🔻
elect your identification method:		-									
🔍 Elemental composition 🔹		í.									
About this method + Download others											
Filter the compounds Using the list below, <u>filter the compounds</u> to show only those whose composition you want to calculate.	Intensity										
Set the calculation parameters Select your composition calculation parameters or create a new parameter set:										I	
Small molecules Edit								1			
Small molecules Edit Calculate the compositions Calculate the compositions will be assigned to the relevant compounds automatically. Calculate the compositions	Legend: 📒 Mat	-	5 120 125 Unmatched fragm	130 135 nent	140 14 m/z		155 16	0 165	170 17	5 180 185	190
Calculate the compositions After the calculation, compositions will be assigned to the relevant compounds automatically. Calculate the compositions	Legend: 🗧 Mat	atched fragment			m/z			0 165	170 175	5 180 185	190
Calculate the compositions After the calculation, compositions will be assigned to the relevant compounds automatically.	Legend: Mat	atched fragment ications: 5 D Description			m/z	Adducts	Formula	0 165	170 175	5 180 185	190
Calculate the compositions After the calculation, compositions will be assigned to the relevant compounds automatically. Calculate the compositions Tag filter applied compounds may be hidden	Legend: Mat Possible identifie Compound ID 49854487	ications: 5 D Description Phenacetin	Unmatched fragm	ient	m/z	Adducts M+H-H		0 165	170 175	5 180 185	190
Calculate the compositions will be assigned to the relevant compounds automatically. Calculate the compositions Tag filter applied compounds may be hidden Edit ompound Accepted ID Tag	Legend: ■ Mat Possible identifie ☆ Compound ID ☆ 49854487 ☆ HMDB31811	ications: 5 D Description Phenacetin 1 3,5-Dimethylph		e	m/z	Adducts M+H-H M+H-H	Formula C10H13NO2	0 165	170 175	5 180 185	190
Calculate the compositions After the calculation, compositions will be assigned to the relevant compounds automatically. Calculate the compositions Tag filter applied compounds may be hidden Edit ompound Accepted ID Tag 17.11.308.1069n	Legend: Mat Possible identifie Compound IE A9854487 HMDB31811 HMDB40021	ications: 5 D Description Phenacetin 1 3,5-Dimethylph	Unmatched fragm enyl methylcarbamat 3-hydroxypropanoyl)	e	m/z	Adducts M+H-H M+H-H M+H-H	Formula C ₁₀ H ₁₃ NO ₂ C ₁₀ H ₁₃ NO ₂	0 165			190
Calculate the compositions After the calculation, compositions will be assigned taily. Calculate the compositions Tag filter applied compounds may be hiden Edit ompound Accepted ID Tag 6.22,308.0856n	Legend: Mat Possible identifie Compound IE A9854487 A9854487 HMDB31811 AHMDB4021 AHMDB41931	atched fragment ications: 5 D Description Phenacetin 1 3,5-Dimethylph 1 2,3-Dihydro-5- 1 3,4-Methyleneod	Unmatched fragm enyl methylcarbamat 3-hydroxypropanoyl)	e -1H-pyrrolizine	m/2	Adducts M+H-H M+H-H M+H-H M+H-H	Formula C10H13NO2 C10H13NO2 C10H13NO2	0 165		5 180 185	190
Calculate the compositions will be assigned to the relevant compositions will be assigned to the relevant compounds automatically. Calculate the compositions will be assigned to the relevant compounds any be hidden Edit Tag filter applied Compound Accepted ID Tag - 7.11_308.1069n	Legend: Mat Possible identifie Compound IE A9854487 A9854487 HMDB31811 AHMDB4021 AHMDB41931	atched fragment ications: 5 D Description Phenacetin 1 3,5-Dimethylph 1 2,3-Dihydro-5- 1 3,4-Methyleneod	Unmatched fragm enyl methylcarbamat 3-hydroxypropanoyl) ioxyamphetamine	e -1H-pyrrolizine	m/2	Adducts M+H-H M+H-H M+H-H M+H-H M+H-H	Formula C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2	0 165			190
Calculate the compositions After the calculation, compositions will be assigned to the relevant compounds' automatically. Calculate the compositions Tag filter applied compounds may be hidden Edit ompound Accepted ID Tag 7.11_308.1069n 6.50_270.1040n 6.50_270.1040n 6.518_1510.058n 6.518_1500.058n 6.518_1500.058n 6.518_1500.058n 6.518_1500.058n	Legend: Mat Possible identifie Compound IE A9854487 A9854487 HMDB31811 AHMDB4021 AHMDB41931	atched fragment ications: 5 D Description Phenacetin 1 3,5-Dimethylph 1 2,3-Dihydro-5- 1 3,4-Methyleneod	Unmatched fragm enyl methylcarbamat 3-hydroxypropanoyl) ioxyamphetamine	e -1H-pyrrolizine	m/z	Adducts M+H-H M+H-H M+H-H M+H-H M+H-H ations: 5	Formula C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2		No stru	icture data	
Calculate the compositions After the calculation, compositions will be assigned to the relevant compounds automatically. Calculate the compositions Tag filter applied compounds may be hidden Edit ompound Accepted ID Tag 6.50, 270.1040n 6.518, 151.0638n 2.91, 151.0638n 0 *	Legend: Mat Possible identifie Compound IE A9854487 A9854487 HMDB31811 AHMDB4021 AHMDB41931	atched fragment ications: 5 D Description Phenacetin 1 3,5-Dimethylph 1 2,3-Dihydro-5- 1 3,4-Methyleneod	Unmatched fragm enyl methylcarbamat 3-hydroxypropanoyl) ioxyamphetamine	e -1H-pyrrolizine	m/z	Adducts M+H-H M+H-H M+H-H M+H-H M+H-H ations: 5	Formula C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2		No stru		Isotope sin
Calculate the compositions Calculate the compositions will be assigned to the relevant compositions will be automatically. Calculate the compositions Calculate the compositions Tag filter applied Calculate the compositions Calculate the compositi	Legend: ● Mat Possible identific ☆ ☆ Compound ID ☆ 49554487 ☆ HMD831811 ☆ HMD84021 ☆ HMD841931 ★ Cl0H13N02	atched fragment ications: 5 D Description Phenacetin 1 3,5-Dimethylph 1 2,3-Dihydro-5- 1 3,4-Methyleneod	Unmatched fragm enyl methylcarbamat 3-hydroxypropanoyl) ioxyamphetamine	e -1H-pyrrolizine elemental con	m/z	Adducts M+H-H M+H-H M+H-H M+H-H M+H-H ations: 5	Formula C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2		No stru	icture data	
Calculate the compositions Calculate the compositions will be assigned to the relevant compositions will be automatically. Calculate the compositions Calculate the compositions Tag filter applied Calculate the compositions Calculate the compositi	Legend: Mat Possible identifie Compound IE A9854487 A9854487 HMDB31811 AHMDB4021 AHMDB41931	atched fragment ications: 5 D Description Phenacetin 1 3,5-Dimethylph 1 2,3-Dihydro-5- 1 3,4-Methyleneod	Unmatched fragm enyl methylcarbamat 3-hydroxypropanoyl) ioxyamphetamine	e -1H-pyrrolizine elemental con Score	m/z nposition identifica Fragment	Adducts M+H-H M+H-H M+H-H M+H-H M+H-H ations: 5	Formula C10H13NO2 C1		No stru Retention	icture data	Isotope sin
Calculate the compositions After the calculation, compositions will be assigned to the relevant compounds automatically. Calculate the compositions Calculate the compositions Calculat	Legend: ● Mat Possible identific ☆ ☆ Compound ID ☆ 49554487 ☆ HMD831811 ☆ HMD84021 ☆ HMD841931 ★ Cl0H13N02	atched fragment ications: 5 D Description Phenacetin 1 3,5-Dimethylph 1 2,3-Dihydro-5- 1 3,4-Methyleneod	Unmatched fragm enyl methylcarbamat 3-hydroxypropanoyl) ioxyamphetamine	e -1H-pyrrolizine elemental con Score A 67.4	m/z e identifica Fragment A 89.2	Adducts M+H-H M+H-H M+H-H M+H-H M+H-H ations: 5	Formula C10H13NO2 C10H13NO		No stru Retention	icture data	Isotope sin 98.30

Note: For the example shown an additional compound ID appears as you accept the identifications based on the calculation of the Elemental Composition.

Scrolling along to the right the actual Mass error and Isotope similarity values are displayed. The **B** indicates the type of search applied.

Note: in this case the only elemental composition returned (using the current thresholds) corresponds to the formula returned for the other possible identifications.

To edit/create/copy a parameter set select from the **Edit** drop down to the right.

2	Set the calculation parameters Select your composition calculation or create a new parameter set:	parameters	
	Small molecules 🔹	Edit 🝷	

As an example create a new parameter set by first copying the existing default set for small molecules.

Select Create a copy... from the Edit drop down, the edit dialog for the calculation parameters will appear.

Vame:	set of ele	emental o	composition ca	lculatio	on para	meter	s that	can be	saved	for lat	er reus	se. Lea	rn mor	re in th	ie <u>onlir</u>	<u>ne refe</u>	rence.				_
Small mo	lecules (2)		н																	He
Compositi	ion:			Li	Be											В	с	N	0	F	Ne
Element	From	То	Remove?																		E
н	0	50	×	Na	Mg											AI	Si	Р	S	CI	Ar
С	0	50	×	к	Ca	Sc	Ti	v	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
N	0	5	×	Rb	Sr	v	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	т	Xe
0	0	20	×	ND.) J	T	21	IND	IVIO	IC.	NU	R11	Pu	Ag	Ca	m	on	30	le	1	~
S	0	2	×	Cs	Ba		Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	TI	Pb	Bi	Po	At	Rr
				Fr	Ra		Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Uut	FI	Uup	Lv	Uus	Uu
						La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Но	Er	Tm	Yb	Lu	
olerance: recursor		e: 3	ppm 🔻			Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr	
sotope si	milarity:	95	%																		

Rename the Parameter set (Small Mol plus P), then add Phosphorous (P) to the composition by clicking on it in the Periodic table.

Then set the maximum number of Phosphates for the composition by adjusting the value in the 'To' column (i.e. 4). Increase the Precursor tolerance to 10ppm.

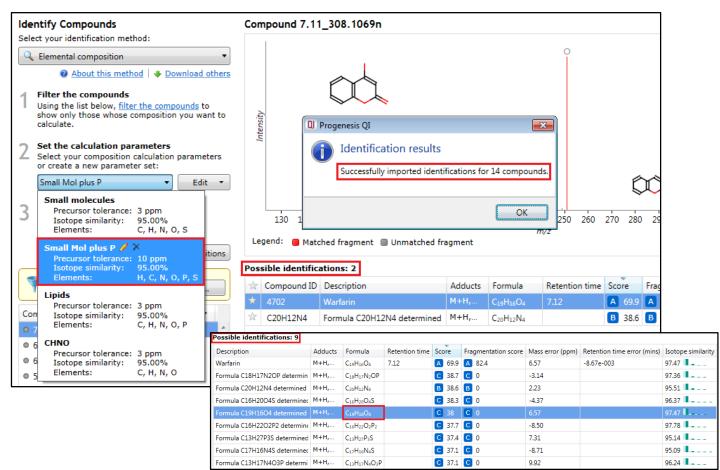
Elemen	tal con	npositio	on calculation p on calculati composition ca	on pa	rame		s that	can be	saved	for lat	er reu:	se. Lea	rn moi	re in th	ıe <u>onlir</u>	<u>ne refe</u>	rence.				
Small Mo	l plus P			н																	He
Compositi	on:			Li	Be											В	с	N	0	F	Ne
Element	From	То	Remove?																		
н	0	50	×	Na	Mg											AI	Si	Р	S	CI	Ar
C	0	50	×	к	Ca	Sc	Ti	v	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Ν	0	5	×				_			-									-		
0	0	20	×	Rb	Sr	Y	Zr	Nb	Мо	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	1	Xe
P	0	4	×	Cs	Ba		Hf	Та	w	Re	Os	Ir	Pt	Au	Hg	ті	РЬ	Bi	Po	At	Rn
S	0	2	×	Fr	Ra		Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Uut	FI	Uup	Lv	Uus	Uuo
								55	9	5				9	0	out		oup		005	000
Tolerance						La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	
Precursor		e: 10	ppm 🔻			Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr	
Isotope si	milarity:	95	%																		
																	Γ	Save		Car	ncel

Finally Save the new parameter set.

Note: on saving, the new parameter set becomes current and its parameters are listed in the drop down.

Make compound 7.11_308.1069n current in the table and click Calculate the composition.

When the compositions are calculated using the adjusted composition and tolerances an additional 7 possible ids appear in the list.



Note: Adding phosphorous to the composition parameter set and increasing the Precursor tolerance returns more possible identifications including the formula for Warfarin which had previously been excluded due to the previously low Precursor tolerance.

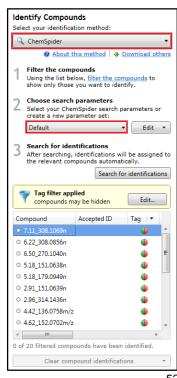
Running a ChemSpider Search

ChemSpider is a web-based chemical structure database with access to over 32 million structures from hundreds of data sources. This method makes use of those ChemSpider web services, automatically exporting data from Progenesis QI to ChemSpider for searching according to the parameters you select, importing the results, and assigning them against the correct compounds within the software.

Note: use of ChemSpider requires internet access and is a service provided to users with a valid Progenesis QI support contract.

Select ChemSpider as the identification method.

If there are already compound identifications present from the previous sections, before performing a search first click on **Clear compound identifications** and click **Clear all identifications**.



Progenesis QI User Guide

ChemSpider can access a large number of data sources when you Search for identifications.

With the search parameters set to Default Click Edit.

The first time you use ChemSpider, KEGG is selected as the default Data Source.

Untick the Perform theoretical fragmentation option

To add additional data sources or change the current data source Click **Select data sources...**.

The ChemSpider data source dialog opens. This enables you to search and select from the 608 data sources currently accessed by ChemSpider.

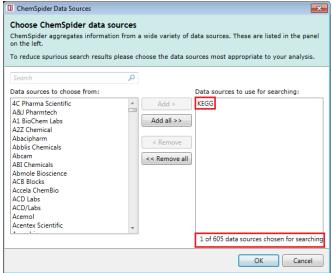
Add the most appropriate source(s) for your current search to the right hand panel.

Tip: since the ChemSpider search is based on accurate mass matching, searching a large number of data sources can take an appreciable amount of time.

The selected Data sources will be added to the Search Parameters. **Note**: if more than five are selected then a number is reported for those used in addition to the 5 displayed.

Then click Search for identifications.

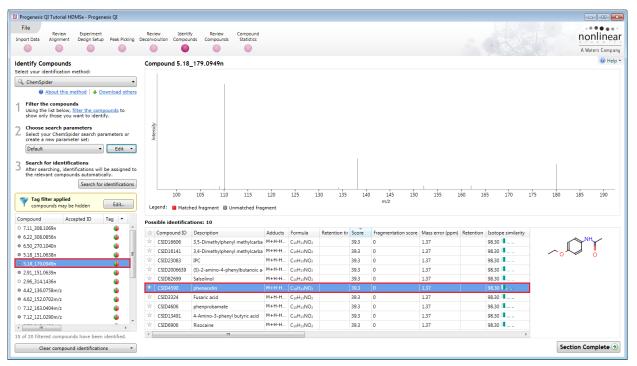
ame: Default		
Required search parame	ters	
Precursor tolerance:	5	ppm
Data source:	KEGG.	Select data sources
Optional search parame	ters	
Perform theoretical f	ragmentation	
Fragment tolerance:	5	ppm
Filter by isotope similar	ilarity score	
Isotope similarity:	95	%
Filter by elemental co	omposition	
Elemental composition	: C: 0-100 H: 0 P: 0-2 S: 0-2	
	Solar	t elemental composition



🔲 Progenesis QI Tutorial HDMSe - Progenesis QI		- • ×
File Review Experiment Import Data Alignment Design Setup Peak Picking	Review Identify Review Compound Deconvolution Compounds Compounds Statistics NO	onlinear
	A Wa	aters Company
Identify Compounds Select your identification method: ChemSpide About this method Abou	Compound 7.11_308.1069n	 Help • Help • 350
Tag filter applied compounds may be hidden Edit Compound Accepted ID Tag • • 7.11_308.1069n • <td>Identification results Successfully imported identifications for 15 compounds. OK No identifications have been imported for this compound</td> <td></td>	Identification results Successfully imported identifications for 15 compounds. OK No identifications have been imported for this compound	
Clear compound identifications	Section Co	mplete 🏵

The progress bar reports the number of masses being searched. When the search completes the number of compounds with imported identifications is displayed.

Note: although Phenacetin is identified as one of the possible identities for **Compound 5.18_179.0949**, based on a mass error tolerance of 5ppm there are 9 other possibilities with the same mass error.



The ChemSpider search parameters includes 3 additional options that can be selected to modify the search:

Theoretical fragmentation can be performed on the search results returned by the ChemSpider search using a specified Fragment tolerance.

Isotope similarity filtering discards ChemSpider results where the isotope similarity score falls below a specified cut-off. **Note**: the score is calculated by comparing theoretical and measured isotope patterns as described for the MetaScope search engine.

Select elemental composition which compares the formula returned by ChemSpider to a specification based on a list of elements and their allowed count ranges.

Q Edit ChemSpider Search	Parameters	X							
ChemSpider search Define a set of ChemSpid reuse. Learn more in the	er parameters that o	can be saved for later							
Name:									
Default									
Required search paramet	ers								
Precursor tolerance:	5	ppm 🔻							
Data source:	KEGG.								
Select data sources									
Optional search parameter	ers								
Perform theoretical fr	agmentation								
Fragment tolerance:	5	ppm							
Filter by isotope simil	arity score								
Isotope similarity:	95	%							
Filter by elemental co	mposition								
Elemental composition:	C: 0-100 H: 0-150 P: 0-2 S: 0-2	N: 0-10 O: 0-30							
	Select elem	ental composition							
	Sa	ave Cancel							

Performing Theoretical Fragmentation on ChemSpider Results

As an example tick the **Perform theoretical fragmentation** option with the **Fragment tolerance** set to 5ppm.

Save the parameter set and re-perform the search.

When the ChemSpider search is re-performed, using the additional information provided by performing theoretical fragmentation on the results this now provides a level of order to the 10 possible identifications for **Compound 5.18_179.0949** making Phenacetin to be the more likely compound identification.

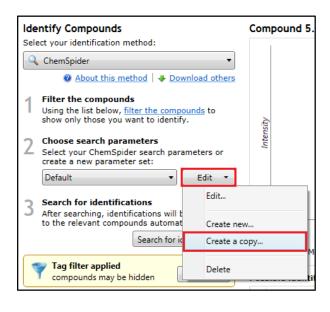
	econvolution Compou	inds Compounds Statistics									nonline
	• •										A Waters Comp
entify Compounds ect your identification method:	Compound 5.18	_179.0949n									() In
		0									
ChemSpider •		Ĭ									
About this method 4 Download others											
Filter the compounds											
Using the list below, filter the compounds to show only those you want to identify.											
Choose search parameters	ţ,					~	/				
Select your ChemSpider search parameters or	tens				^	11					1 × 1
create a new parameter set:	5					~					
Default • Edit •									~		
Search for identifications						1			\sim	Π	
After searching, identifications will be assigned to											
the relevant compounds automatically.		0						0			
Search for identifications		<u></u>						. I			
😴 Tag filter applied	100	105 110 115	120	125	130 135	14	0 145 m/z	150 155	160	165 170	175 180 185 190
compounds may be hidden Edit	Legend: 😑 Matc	hed fragment 🔳 Unmatched frag	ment				m/z				
ompound Accepted ID Tag 🔻											
7.11_308.1069n	Possible identifica					*					
6.22 308.0856n	🚖 Compound ID		Adducts		Retention tir Sc	ore i	Fragmentation score	Mass error (ppm)			5 NU
	* CSID4590	phenacetin	M+H-H	C10H13NO2	В	56.8	B 87.1	1.37		8.30	
6.50 270.1040n	* CSID2006639	(S)-2-amino-4-phenylbutanoic a	M+H-H	C10H13NO2	В		B 54.2	1.37		8.30	
· · · · ·	M CSID2000039							1.37	9	8.30	- 0 - 0
5.18_151.0638n 🕢 🗉	☆ CSID2006839	3,4-Dimethylphenyl methylcarba	M+H-H	C10H13NO2	В	49.9	B 52.7	1.57			
5.18_151.0638n 🔮 🗉		1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1			_		B 52.7 B 52.6	1.37		8.30	
5.18_151.0638n	* CSID16141	3,4-Dimethylphenyl methylcarba 3,5-Dimethylphenyl methylcarba	M+H-H		B	49.9	_		9		
5.18_151.0638n	 ☆ CSID16141 ☆ CSID16606 	3,4-Dimethylphenyl methylcarba 3,5-Dimethylphenyl methylcarba	м+н-н м+н-н	C10H13NO2	B	49.9 49.6	B 52.6	1.37	9 9	8.30	
5.18_151.0638n 5.18_179.0949n 291_151.0639n 295_134.136n 442_136.0758m/z	 ☆ CSID16141 ☆ CSID16606 ☆ CSID13491 	3,4-Dimethylphenyl methylcarba 3,5-Dimethylphenyl methylcarba 4-Amino-3-phenyl butyric acid	M+H-H M+H-H M+H-H	C10H13NO2 C10H13NO2	B	49.9 49.6 49.5	B 52.6 B 51.3	1.37 1.37	9 9 9	8.30	
5.18_179.0949n	☆ CSID16141 ☆ CSID16606 ☆ CSID13491 ☆ CSID82699	3,4-Dimethylphenyl methylcarba 3,5-Dimethylphenyl methylcarba 4-Amino-3-phenyl butyric acid Salsolinol	M+H-H M+H-H M+H-H M+H-H	C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2	B	49.9 49.6 49.5 49	B 52.6 B 51.3 B 51	1.37 1.37 1.37	9 9 9	8.30	
5.18_151.0638n	☆ CSID16141 ☆ CSID16606 ☆ CSID13491 ☆ CSID82699 ☆ CSID4606	3,4-Dimethylphenyl methylcarba 3,5-Dimethylphenyl methylcarba 4-Amino-3-phenyl butyric acid Salsolinol phenprobamate	M+H-H M+H-H M+H-H M+H-H	C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2 C10H13NO2	8 8 8 8 8	49.9 49.6 49.5 49 49	B 52.6 B 51.3 B 51 B 48.4	1.37 1.37 1.37 1.37 1.37	9 9 9 9	8.30	
5.13_151.0638n	☆ CSID16141 ☆ CSID16006 ☆ CSID13491 ☆ CSID82699 ☆ CSID4606 ☆ CSID4606 ☆ CSID23083	3,4-Dimethylphenyl methylcarba 3,5-Dimethylphenyl methylcarba 4-Amino-3-phenyl butyric acid Salsolinol phenprobamate IPC Fusaric acid	M+H-H M+H-H M+H-H M+H-H M+H-H	$\begin{array}{c} C_{10}H_{13}NO_2\\ C_{10}H_{13}NO_2\\ C_{10}H_{13}NO_2\\ C_{10}H_{13}NO_2\\ C_{10}H_{13}NO_2\\ C_{10}H_{13}NO_2 \end{array}$	8 8 8 8 8	49.9 49.6 49.5 49 49 49 42.6	 B 52.6 B 51.3 B 51 B 48.4 B 48.4 	1.37 1.37 1.37 1.37 1.37 1.37	9 9 9 9 9 9	8.30 .	

Filtering using ChemSpider Results using Elemental composition

To apply a filter based on a specified elemental composition to ChemSpider you must first set up the filter as a new or edited parameter set.

Tip: a simple way to create a new search parameter set is to **Create a copy**, rename and edit an existing set.

With the Default set as current, select **Create a copy** from the Edit menu. In the parameters dialog rename this set (i.e. CHN Filter) and tick **Filter by elemental composition** and untick Perform theoretical fragmentation.



Required search parameters	
Precursor tolerance: 5 ppm	
Data source: KEGG.	
Select data	sources
Fragment tolerance: 5 ppm	
Isotope similarity: 95 %	
Filter by elemental composition	
Elemental composition: C: 0-100 H: 0-150 N: 0-10 0	0: 0-30

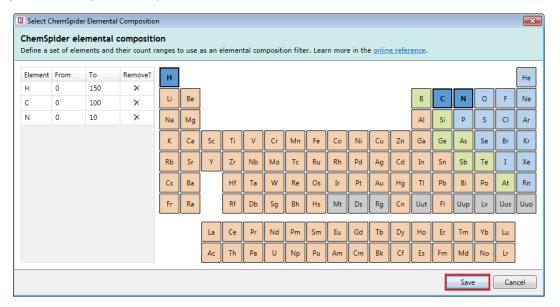
Click **Select elemental composition...**to edit the current Elemental composition.

The existing composition is displayed.

Element	From	То	Remove?	н																	Не
н	0	150	×																		
с	0	100	×	Li	Be											В	C	N	0	F	Ne
N	0	10	×	Na	Mg											AI	Si	Р	s	CI	Ar
0 p	0	30 2	×	К	Ca	Sc	Ti	v	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
5	0	2	×	Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
				Cs	Ba		Hf	Та	w	Re	Os	Ir	Pt	Au	Hg	TI	Pb	Bi	Po	At	Rn
				Fr	Ra		Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Uut	FI	Uup	Lv	Uus	Uu
						La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Но	Er	Tm	Yb	Lu	
						Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr	

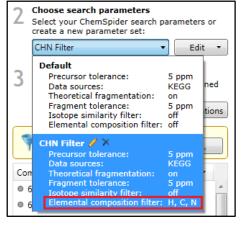
Remove elements either by clicking on the x in the table or on the element(s) in the periodic table. Then adjust the ranges (From and To) of atoms for each element in the formula using the table.

Finally **Save** the adjusted composition.



The amended composition appears in the CHN Filter parameter set. Save this to make it current.

The new set now displays the Elemental Composition Filter



As an example using the Default search parameters ChemSpider returns 10 possible identities for **Compound 2.46_315.1496**

2 Choose search parameters Select your ChemSpider search parameters reate a new parameter set: Default 3 Search for identifications After searching, identifications will be assig the relevant compounds automatically. Search for identific	ned to			This	5 compour	nd has no	fragm	entation data		
Tag filter applied Edit										
Compound Accepted ID Tag	P	ossible identificat	tions: 10							
© 2.91_151.0639n	^	Compound ID	Description	Adducts	Formula	Retention tir	Score	Fragmentation score	Mass error (ppm)	
o 2.96_314.1436n			(2E)-2,3-Diphenyl-3-(2-pyridinyl)				36.2	0	1.36	
• 4.42_136.0758m/z			(7beta,17R,19E,21alpha)-21-Hyd				36	0	1.04	
© 4.62_152.0702m/z	- E							-		
o 7.12_163.0404m/z			(1R,10S,12R,13R,14S,16S,18R)-13				36	0	1.04	
• 7.12_121.0290m/z	-	CSID391595	Methyl (19alpha)-19-methyl-16,1				36	0	1.04	Í Ý 🔪
© 2.75_315.1496m/z		CSID4445235	Methyl (15alpha,19E)-16-formyls				36	0	1.04	N
○ 2.97_190.1357m/z		CSID391596	Methyl (19beta)-19-methyl-16,1				36	0	1.04	
• 0.63_320.1087m/z	2	CSID4573616	Methyl (19E)-16-formylsarpagan	M+H-2	$C_{21}H_{22}N_2O_3$		36	0	1.04	
• 2.46_315.1496m/z	2	CSID2272523	(E)-Ranitidine	M+H	$C_{13}H_{22}N_4O_3S$		35.9	0	3.26	
• 3.67_368.1013m/z	2	CSID77849	Aphistar	M+H	$C_{13}H_{22}N_4O_3S$		35.9	0	3.26	
· · · · · · · · · · · · · · · · · · ·	F 3	CSID10128463	Testosterone, 9-chloro-17-methy	M+H-2	C20H27CIO3		31.5	0	-4.18	
15 of 20 filtered compounds have been identifie	d. ·	(÷	
Clear compound identifications	-									Section Complete 🦻

Clear the compound identifications and change the search parameters to CHN Filter and click **Search for identifications**.

2 Choose search parameters Select your ChemSpider search parameters or create a new parameter set: CHN Filter • Edit •	This compound has no fragmentation data
3 Search for identifications After searching, identifications will be assigned to the relevant compounds automatically. Search for identifications	
Tag filter applied compounds may be hidden Edit	
Compound Accepted ID Tag 💌	Possible identifications: 1
○ 2.91_151.0639n	Compound ID Description Adducts Formula Retention tir Score Fragmentation score Mass error (ppm)
○ 2.96_314.1436n	* CSID2274171 (2P-33-Diphenyl-3-(2-pyridimyl) (M+CH3-, CayHuM2) 362 0 1.36
• 4.42_136.0758m/z	* Calozzrazia (cc)-c-orphienty-s-(c-pynomy) (************************************
○ 4.62_152.0702m/z	
o 7.12_163.0404m/z	
0 7.12_121.0290m/z	I I N
© 2.75_315.1496m/z	N 1
○ 2.97_190.1357m/z	
○ 0.63_320.1087m/z	
• 2.46_315.1496m/z	
○ 3.67_368.1013m/z	
· · · · · · · · · · · · · · · · · · ·	
4 of 20 filtered compounds have been identified.	* <u> </u>
Clear compound identifications •	Section Complete

Application of the filter to the ChemSpider results reduces the possible identities to one based on restricting the composition to CHN.

Running a LipidBlast Search

As the samples for this tutorial were prepared from Urine, using LipidBlast as your primary method of compound identification is not appropriate.

For details on how to setup and use LipidBlast refer to Appendix 6 (page 98)

Running a NIST MS/MS Search

Select NIST MS/MS library from the Identifications Methods.

Note: to use this compound search method you will need to purchase the NIST MS/MS library. Hence using this section is dependent on you having purchased and downloaded the NIST MS/MS library plugin.

The NIST library search plugin bundles the NIST MS/MS libraries and performs a combination of neutral mass and MS/MS based searches. It uses the same search and scoring algorithms as <u>Progenesis</u> <u>MetaScope</u>.

Firstly, delete all your compound identifications using "Clear compound identifications".

Set the required search parameter for the both the Precursor and the Fragment mass error tolerances for matching each compound and its observed fragments, the default tolerance is 5ppm.

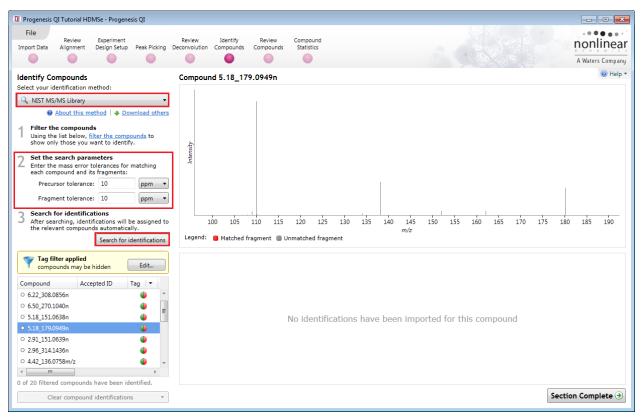
Precursor tolerance

This value, in ppm or Da, is the maximum difference between the mass for the compound in the NIST library and the observed mass.

Fragment tolerance

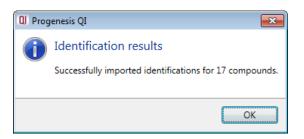
This threshold (in ppm or Da) is the maximum difference between the mass of a fragment in the NIST MS/MS library and the observed mass of the fragment.

For this example set both tolerances to 10ppm.



Then click Search for identifications.

The progress bar reports the number of masses being searched. When the search completes the number of compounds with imported identifications is displayed.



You can visualise the matching of measured to database-predicted fragments for each potential ID using the 'mirror plot' on the same screen, after clicking on a search result. Matched fragments are shown in red, unmatched in grey; and hovering the cursor over a fragment match will display relevant information in a tooltip.

QI Progenesis QI Tutorial HDMSe - Progenesis QI		
File Review Experiment	Review Identify Review Compound	nonlinear
Import Data Alignment Design Setup Peak Picking	Deconvolution Compounds Compounds Statistics	D V N A M T C S
		A Waters Company
Identify Compounds	Compound 5.18_179.0949n	🔞 Help 🔻
Select your identification method:		
NIST MS/MS Library About this method + Download others	σ	
	Measured	
Filter the compounds Using the list below, filter the compounds to	We	
show only those you want to identify.	Peak m/z: 110.0608 Intensity: 3824.03 (100.00%)	
2 Set the search parameters Enter the mass error tolerances for matching	Database m/z: 110.0601 A m/z: 0.0007	
each compound and its fragments:		
Precursor tolerance: 10 ppm 💌	Database	
Fragment tolerance: 10 ppm 🔹	ă	
2 Search for identifications		
After searching, identifications will be assigned to the relevant compounds automatically.	100 105 110 115 120 125 130 135 140 145 150 15 m/z	5 160 165 170 175 180 185 190
Search for identifications	Legend: 🗧 Matched fragment 🔲 Unmatched fragment	
Tag filter applied	Possible identifications: 1	
compounds may be hidden	☆ Compound ID Description Adducts Formula Retention tir Score Fragmentation score	Mass error (ppm)
Compound Accepted ID Tag 💌	★ 62-44-2 Phenacetin M+H-H C ₁₀ H ₁₃ NO ₂ 58.8 97.3	
○ 6.22_308.0856n		
● 6.50_270.1040n		Y NH
• 5.18_151.0638n		
© 2.95 314.1436n		
• 4.42_136.0758m/z		
< >		
17 of 20 filtered compounds have been identified.	۲	•
Clear compound identifications -		Section Complete 🏵

For all the identification methods described in this section additional information and guidance can be obtained **online** in the form of FAQs accessed by the links imbedded in the software.

QI Progenesi	s QI Tutorial HE)MSe - Progene	sis QI		
File	Daviaw	Franciscont		Deview	Ideatif.
Import Data	Review Alignment	Experiment Design Setup	Peak Picking	Review Deconvolution	Identify Compounds
				_	
-	ompounds identification r	method:		Compoun	id 5.18_17
Select your		method:	×	Compoun	id 5.18_17
Select your	identification r IS/MS Library	method:	vnload others		id 5.18_17

For each identification method the **About this method** link will take you to relevant page(s) for the current method.

Stage 9: Review Compounds

The Review Compounds stage allows you to examine the behaviour of all or subsets of compounds based on tag filters.

Note: having explored the various methods for identifying compounds, before you use **Review Compounds** return to the Identify Compounds stage and click **Clear compound identifications (if not already clear)**, **select to clear all identifications**, then select Progenesis MetaScope as the identification method, select Tutorial Theoretical Fragmentation as the current search parameters and check they are set as shown below using the **Edit** drop down.

Q Progenesis QI Tutorial HDMSe - Progenesis QI			
File Import Data Review Alignment Design Setup Peak Picking	Review Identify Review Deconvolution Compounds	Edit Search Parameters MetaScope search parameters Define a set of MetaScope parameters that can be saved for later	A Waters Company
Identify Compounds Select your identification method: Progenesis MetaScope • About this method ◆ Download others 1 1 Filter the compounds 0 1 Select your MetaScope search parameters or create a new parameter set: Itutorial Theoretical Fragmentati ◆ Edit ◆ 3 Search for identifications Identifications will be assigned to the relevant compounds automationally. Search for identifications Identifications may be hidden Edit Y and Theoretical Fragmentati ◆ Edit Compound Accepted ID Tag ◆ 0 6.22, 308.0856n 0 5.18, 151.0638n 0 2.96, 314.1436n 0 4.24, 21.36.0758m/z 0 6.42, 151.072m/z 0 0 f 20 filtered compounds have been identified.	Compound 5.18_179.0949n	Define a set of indexiduple parameters that can be saved for later reuse. Learn more in the online reference. Name: Tutorial Theoretical Fragmentation Compound database CAProgram Files (x86)\Nonlinear Dynamics\Prog Browse Data format: Additional compound properties source Retention time within: 0.1 minutes CCS within: 25.9 Additional compound properties source Read additional compound properties from this file CAUsers\andy.borthwick\Documents\Customer [Browse Fragment search method O no to use fragmentation data @ Perform theoretical fragmentation Fragment tolerance: 12 Preament tolerance: 12 Pragment tolerance: 12 ppm * Perform fragment database search * CAUsers\andy.borthwick\Documents\Customer [Browse Pragment tolerance: 12 ppm * Perform fragment database search CAUsers\andy.borthwick\Documents\Customer [Browse Fragment tolerance: <th>Help •</th>	Help •
Clear compound identifications 👻		S	ection Complete 🏵

Click **Search for identifications.** Click **Edit** on the applied tag filter the tags created earlier should still be applied, if not set them up as described in tagging page 36. The display should look as below.

I Progenesis QI Tutorial HDMSe - Progenesis QI									
File				ſ	Q Filter the c	ompounds			
Import Data Alignment Design Setup Peak Picking	Review Deconvolution	Identify Compounds	Compounds	Compour Statistic	Create a f	ilter e compounds			eir tags. Move tags to the appropriate boxes
Identify Compounds	Compound	d 5.18_1	79.0949n (Ph	enaceti	Available tac		-		how compounds that have all of these tags:
Select your identification method:			0		Available tag	15:			Anova p-value ≤ 0.05 (1258 compounds)
Progenesis MetaScope			Ĭ						Max fold change ≥ 2 (450 compounds)
About this method Download others									· · · · · · · · · · · · · · · · · · ·
Filter the compounds									how compounds that have at least one of hese tags:
Using the list below, <u>filter the compounds</u> to show only those you want to identify.	2							1	
Choose search parameters	Intensity								
Z Select your MetaScope search parameters or	Int								lide compounds that have any of these tags:
create a new parameter set:									Not identified (5082 compounds)
Tutorial Theoretical Fragmentati 🔹 Edit 🔹									
2 Search for identifications									
Identifications will be assigned to the relevant compounds automatically.		0	29		Clear the filt	tar			OK Cancel
Search for identifications	10	0 105	110 115 1	20 12	Clear the fill	ter			OK Cancer
	Legend:	Matched	fragment 🔳 Uni	matched f	ragment	•			
Tag filter applied compounds may be hidden Edit	Possible ide	entificatio	ns: 4						
Compound Accepted ID Tag 💌	🚖 Compo	und ID D	escription	Adducts	Formula	Retention tir	Score	Fragmentation	score
• 7.11_308.1069n	対 498544	187 PI	henacetin	M+H-H	C10H13NO2	5.16	67.4	89.2	
● 6.22_308.0856n	THE HMDB	31811 3,	5-Dimethylphenyl	M+H-H	C10H13NO2		49.8	52.6	
• 6.50_270.1040n	THMDB4		3-Dihydro-5-(3-h)				42.8	17.6	
• 5.18_151.0638n	THMDB4	41931 3,	4-Methylenedioxy	M+H-H	C10H13NO2		41.6	11.4	
5.18_179.0949n 49854487									
• 2.91_151.0639n									
• 2.96_314.1436n									
• 4.42_136.0758m/z									
All 20 filtered compounds have been identified.	•								•
Clear compound identifications -									Section Complete $ e e e e e e e e e e e e e $

Finally to accept Phenacetin as **Compound 5.18_179.0949n's** identity, click on the 'star' to the left of the Compound ID.

Now move to Review Compounds by clicking on the Workflow.

File Review Experiment	Review Identify	Review	Compo		1					nonline	•
port Data Alignment Design Setup Peak Picking	Deconvolution Compound	ds Compound	s Statis	ics)						A Waters Comp	C
eview Compounds	F 1 F		2		C 10		 Filter is a 			@ He	
ing this screen, you can find the compounds of	Find a compound: Sea				Filter com						
terest in your experiment.	Compound	Neutral mass		z	Retention time			Tag 🔹 💌	Accepted ID	Identifications	
Create a shortlist to review	• 7.11_308.1069n	308.1069	347.0700	1	7.11	3.40	0.18	4		1	-
In the table, sort and <u>filter the compounds</u> based on their measurements, to generate a shortlist	6.22_308.0856n	308.0856	309.0929	1	6.22	3.13	0.30	•		1	
for further review.	• 5.18_179.0949n	179.0949	180.1043	1	5.18	2.05	0.18	٩	49854487	4	l
How are the measurements calculated?	• 2.96_314.1436n	A 436	315.1509	1	2.96	3.02	0.27	4		1	l
To sort the table by a given value, simply click the relevant column header.	4.08_235.1822m/z	nown>	235.1822	1	4.08	2.54	0.18	4		1	
	• 3.69_195.0897m/z	<unknown></unknown>	195.0897	1	3.69	1.94	0.16	4		2	
Review the compounds For each compound of interest, inspect the ions'	• 7.12_163.0404m/z	<unknown></unknown>	163.0404	1	7.12	1.62	0.17	4		1	
alignment and peak picking:	• 2.91_151.0639n	151.0639	152.0707	1	2.91	1.67	0.15	4		4	
Review selected compound	6.50_270.1040n	270.1040	271.1113	1	6.50	2.75	0.14	4		1	
Yo B so double-click to review a compound.	•									•	
so double-click to review a compound.	Compound 5.18 179.0	949n:									
Choose the correct identifications For each compound, select one of its possible identifications as the accepted one.	Compound abundanc	e Possible ide	entifications	3	D Montage D	rift time mo	ntage				
To speed this up, you can automatically accept		C_1	Norm			A_LD			B_HD		
identifications in compounds where only one of the possible identifications has:	10.866667 -								•		
Score ≥ 50.0 Accept identifications	ndanc					} ⊕ €					
Export data for further processing By exporting your data to external tools, there's no limit to your analysis.											
Export to pathways tool	- Nor					С					
	- ArcSin 0.200000 -	Ĩ									
	1										
periment design view vour data from a different perspective:	4									×.	

Window A displays the main table of Compounds with identifications as well as those that remain **unknown** after the identifications have been imported.

Window B Provides tools to set thresholds for the acceptance of identifications based on a score.

Window C displays either: a Compound abundance plot, list of Possible identifications, 3D Montage, Drift Time Montage (for data collected with drift time), for the current compound highlighted in Window A.

Using **Compound 5.18_179.0949n** as an example the 4 views for Window C show:

Compound abundance	Possible identifications	3D Montage	Drift time montage	
	C_Norm		A_LD	B_HD
10.8666667			M	M
5.533333 - 				
0.200000 - -	B			

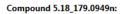
The **Compound Abundance** displaying normalised values for each run, a mean value and 3 standard deviations.

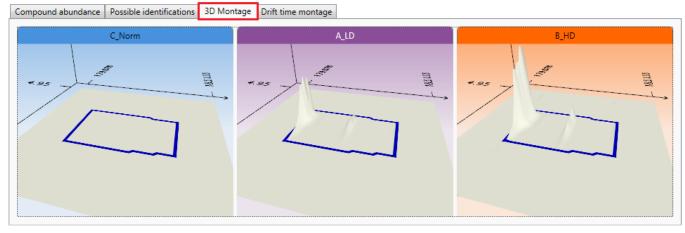


Possible identifications for current compound with Overall Score based on, Mass error, Retention Time (if available), Isotope Similarity based on the comparison of the measured isotope distribution for the compound vs the expected based on the compound formula, Fragmentation Score and CCS if available.

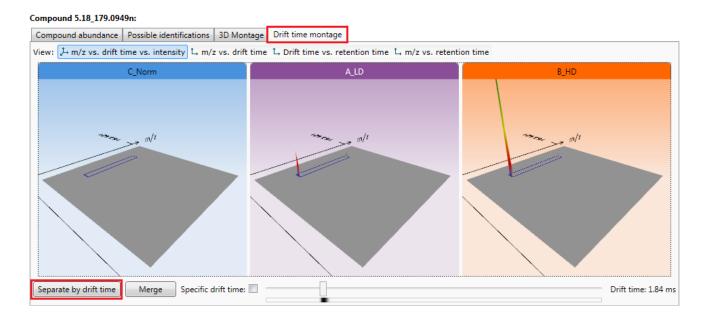
Con	npound abunda	nce Possible identifications	3D Mon	tage Drift f	time montage			
os	sible identific	ations: 4						
Å	Compound ID	Description	Adducts	Formula	Retention time	Score	Fragmentation score	
☆	49854487	Phenacetin	M+H-H	C ₁₀ H ₁₃ NO ₂	5.16	67.4	89.2	
÷	HMDB31811	3,5-Dimethylphenyl methyl	M+H-H	C ₁₀ H ₁₃ NO ₂		49.8	52.6	
÷	HMDB40021	2,3-Dihydro-5-(3-hydroxyp	M+H-H	C ₁₀ H ₁₃ NO ₂		42.8	17.6	∕ ₀′ ❤ 0
÷	HMDB41931	3,4-Methylenedioxyamphe	M+H-H	C10H13NO2		41.6	11.4	

The **3D montage** is based on the first run in each group.





The Drift time montage tab is only shown for HDMSe data.



Setting a Compound's identity

If you have not already set or accepted a compounds identity at the **Identify Compounds** stage, or wish to amend it as a result of reviewing the data displayed here then you have the same functionality available to you in the **Possible Identifications** tab

For example to accept Warfarin as the identity for Compound 7.11_308.1069n click on the 'star'.

This updates the compound's 'Accepted ID' and Description.

File Review Experiment Import Data Alignment Design Setup Peak Picking	Review Deconvolution	Identify Compounds	Review Compounds	Compoun Statistics	đ					n	online
										A	Waters Comp
Review Compounds	Find a com	ound: Searc	h	,P	Filter comp	ounds 🔻 Fil	ter is active				🕜 He
Using this screen, you can find the compounds of interest in your experiment.	Tag 💌	Accepted ID	Identifications	Anova (p)	Max fold change	Highest mean	Lowest mean	Isotope distribution	Max Abundance	Min CV%	Description
Create a shortlist to review			1	< 1.1E-16	1.5E+05	B_HD	A_LD		17281.5107	4.65	
In the table, sort and filter the compounds based			1	< 1.1E-16	5.2E+04	B HD	C Norm		3742.5726	1.09	
on their measurements, to generate a shortlist for further review.		49854487	4	< 1.1E-16	4.32E+04	B HD	C Norm		12184.7590	1.88	Phenacetin
Mow are the measurements calculated?			1	< 1.1E-16	4.91E+03	B_HD	C_Norm		275.8854	1.69	
To sort the table by a given value, simply click			1	< 1.1E-16	3.33E+03	B_HD	C_Norm		1169.9322	3.88	
the relevant column header.	۵	4702	1	< 1.1E-16	1.48E+03	B_HD	C_Norm		18742.8091	2.93	Warfarin
7 Review the compounds	•		2	< 1.1E-16	1.32E+03	B_HD	C_Norm		6191.8644	2.70	
For each compound of interest, inspect the ions' alignment and peak picking:			1	< 1.1E-16	815	B_HD	C_Norm		11793.3170	4.37	
Review selected compound			1	1.11E-16	239	B_HD	A_LD		95.7501	7.47	
You can also double-click to review a compound.			1	1.97E-14	199	B HD	A LD		44.0154	8.80	
			2	3 96F-13	147	R HD	C Norm		495 7524	1.60	
3 Choose the correct identifications For each compound, select one of its possible identifications as the accepted one.	Compound	7.11_308.10	69n:								•
To speed this up, you can automatically accept	Compoun	d abundance	Possible identi	ifications	BD Montage Drift	t time montage					
identifications in compounds where only one of the possible identifications has:	Possible	identificatio	ns: 1								
Score ≥ 50.0 Accept identifications	🚖 Com	bound ID De	scription	Ad	Iducts Formula	Retention f	time Score Fr	agmentation scor			
	* 4702	. Wa						2.4			
4 Export data for further processing By exporting your data to external tools, there's no limit to your analysis. Export to pathways tool											
Experiment design Review your data from a different perspective:	•							Þ		~ _0	
Current design: Minus QC 🔹										Section C	omplete

Rather than review all the possible compound identifications you can set a threshold for the score. This allows the automatic acceptance of compound identity where **only** one of the possible identifications is greater than the defined score.

As an example set the threshold as 48 and then order on Accepted ID and Description.

File Review Alignment	Experiment Design Setup Peak Picking	Review Deconvoluti	Identify on Compound	Review ds Compounds	Compo s Statist						~/	nonline
					•							A Waters Comp
Review Compounds		Find a co	mpound: Sea	rch	p	Filter co	mpounds 💌	Filter is active				🕜 He
Jsing this screen, you ca nterest in your experime	n find the compounds of nt.	ag 🔻	Accepted ID	Identifications	Anova (p)	Max fold change	Highest mean	Lowest mean	Isotope distribution	Max Abundance	Min CV%	Description
Create a shortlist	to review	-	14708992	1	< 1.1E-16	4.91E+03	B_HD	C_Norm		275.8854	1.69	Acetanilide
	filter the compounds based		7847292	1	< 1.1E-16	1.5E+05	B_HD	A_LD		17281.5107	4.65	Alprazolam
for further review.	nts, to generate a shortlist	4	46511425	2	< 1.1E-16	1.32E+03	B_HD	C_Norm		6191.8644	2.70	Caffeine
How are the end of	e measurements calculated?		855682	1	< 1.1E-16	Infinity	B_HD	C_Norm	1	8749.4568	2.58	Lidocaine
	a given value, simply click		46506142	4	< 1.1E-16	86.5	B_HD	C_Norm		1290.3892	2.00	Paracetamol
the relevant column	neader.		49854487	4	< 1.1E-16	4.32E+04	B_HD	C_Norm		12184.7590	1.88	Phenacetin
Review the compo			7847488	1	< 1.1E-16	815	B_HD	C_Norm		11793.3170	4.37	Ranitidine
 For each compound alignment and peak 	of interest, inspect the ions' picking:		9357	1	< 1.1E-16	3.33E+03	B_HD	C_Norm		1169.9322	3.88	Tolbutamide
	Review selected compound	۹	4702	1	< 1.1E-16	1.48E+03	B_HD	C_Norm		18742.8091	2.93	Warfarin
Yeu ere also double	click to review a compound.			1	1.97E-14	199	B_HD	A_LD		44.0154	8.80	
Tou can also double.	click to review a compound.			2	3 96F-13	147	R HD	C Norm		495 7524	1.60	
identifications as the To speed this up, yo	select one of its possible accepted one. u can <i>automatically</i> accept npounds where only one of	Compo	nd 3.69_195.0 und abundanc le identificat	e Possible ide	ntifications	3D Montage	Drift time monta	ge				
Score ≥ 48.0	Accept identifications	🚖 Co	mpound ID	Description		Adducts Formu	la Retentio	on time Score	Fragmentation		0	
50010 E 40.0	Accept Identifications	📩 46	5511425 C	affeine		M+H C8H10N	I ₄ O ₂ 3.69	71.2	82.9		Ĭ	1
Export data for fur By exporting your da no limit to your anal	ata to external tools, there's	☆ H	MDB12488 1	,2,3,4-tetrahydro	o-beta-car	M+Na C ₁₁ H ₁₂	N ₂	38.8	0	N		
xperiment design	different perspective:	٠							•		I	

Note: accepting a score threshold causes the Accepted ID and Description fields to be populated when there is a single ID with a score \geq 48 for the compound.

In this example Paracetamol (2.91_151.0638n) and Phenacetin (5.18_179.0949) were accepted manually. Also the identification of Aspirin (7.12_163.0404) was rejected by clicking on the star icon for possible identifications.

Compound Validation View

Further information about a compound and its adduct forms, which enables you to confirm the validity of the measurements, is available in the Compound Validation View.

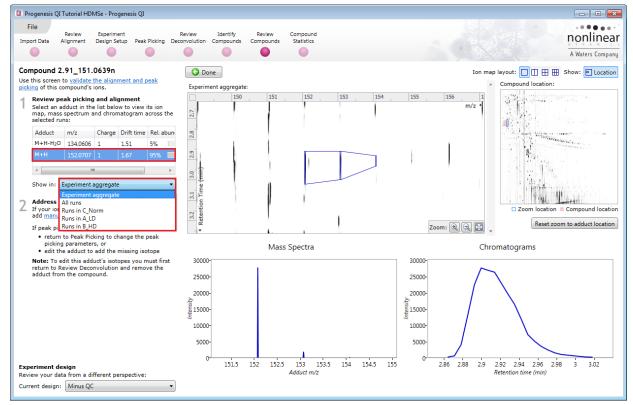
In this view:

- A montage of ion maps helps to validate the ions' peak picking and alignment
- Graphs of mass spectra and chromatograms provide further confirmation of correct peak picking

To open the view either double click on the Compound in the Review Compounds screen or click on Review selected compound

Try double clicking on the compound **2.91_151.0638n** (note: you can accept this compound's identity, as Paracetamol by clicking on the 'star' in the table)

As the **Compound Validation** view opens you can select which adduct of the compound to review first, in this example (M+H).



Use the **Show Location** option at the top right of the screen to view the ion's location within the full ion map. **Note**: you can 'toggle' this view on and off and reset the zoom for the adduct location.

Below the list of adducts, a drop-down list lets you select whether you want to see the selected adduct:

- on the experiment aggregate
- across all runs, or
- only on runs within a single experiment condition

The **Experiment aggregate** option is most useful for validating the ions' peak picking, as the aggregate is generated immediately prior to peak picking and is used as the input to that process. The aggregate pattern of detection can be edited to add a missing isotope using the **Edit adduct** facility.

Note: details on Editing adducts is available in Appendix 7 (page 100)

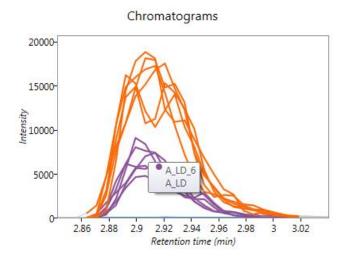
To validate the alignment you will find it best to select to select the **All runs** option and then adjust the ion map layout to display multiple runs



Use the lon map layout and zoom tools to set up the views as shown above. The multi-panels allow you to confirm the alignment across all the runs.

Note: that when the **All runs** are selected the mass spectra and chromatograms are shown for all the runs in the experiment.

In the chromatograms view each line corresponds to a single run, coloured according to the experiment condition to which it belongs. If you hover the cursor over a line, the name of the run which generated it will appear. When showing all runs, these graphs give further validation of how the adduct form's abundance is changing between experimental conditions.



Compounds separated by Drift Time

To view compounds separated by drift time, first clear the existing Filter by clicking on **Filter compounds** and then **Clear the filter.**

Then right click on the Review Compounds Table and create a Quick Tag for those compounds Separated by drift time.

Re	view Compounds	Find a compound: Search				P Filter compounds 👻											
	ng this screen, you can find the compounds of rest in your experiment.	Compoun	d	Neutral mass	m/z	z	Retention time	Drift time	Peak Width	Tag 💌	Accepted ID	Identifications	Anova (p)	N			
1	Create a shortlist to review	• 5.18_179.0949n 179.0949		179.0949	180.1043	1	5.18	2.05	0.18	۲	49854487	4	< 1.1E-16	6 _			
	In the table, sort and <u>filter the compounds</u> based on their measurements, to generate a shortlist	• 3 🧉	Anova p-v	alue ≤ 0.05	95.0897	1	3.69	1.94	0.16	۹	46511425	2	< 1.1E-16	1			
	for further review.	• 2 🕥	Max fold c	hange ≥ 2	52.0707	1	2.91	1.67	0.18	۲	46506142	4	< 1.1E-16	6			
	How are the measurements calculated?	• 4 🔵	Not identif	ied	36.0758	1	4.42	1.57	0.14	۲	14708992	1	< 1.1E-16	6			
	To sort the table by a given value, simply click the relevant column header.	• 2			15.1509	1	2.96	3.02	0.27	۲	7847488	1	< 1.1E-16	8			
	the relevant column header.	New tag Quick Tags			nov	a p-value			۲	7847292	1	< 1.1E-16	1				
2	Review the compounds	• 4 👝	• 4		-		fold change			۲	855682	1	< 1.1E-16	Ir			
~	For each compound of interest, inspect the ions' alignment and peak picking:	• 6					num CV			۲	9357	1	< 1.1E-16	3			
	Review selected compound	• 7.11_3		308.1009	- N	lot i	dentified			۲	4702	1	2.22E-16	1			
	You can also double-click to review a compound.	 7.11_308.1009n 308.1009 4.96_134.0966m/z <unknown></unknown> 1 		1 N	lot f	ragmented			•		0	0.331	1				
		0 4.96_696.2684m/z <unknown> 1</unknown>		6 S	epai	rated by drift tim	e		•		0	0.319	1 -				
3	Choose the correct identifications For each compound, select one of its possible	○ 4.96_696.2684m/z <unknown> 6</unknown>			Ic	lent	ified and separat	ed by drift t	ime					F			

Once you have created the tag then filter the table to show only those compounds Separated by Drift Time.

QI Filter the compounds Create a filter Show or hide compounds based on a selection	of their tags. Move tags to the appropriate
boxes to create the filter. For more guidance, p Available tags:	Show compounds that have all of these tags:
 Not identified (5082 compounds) Anova p-value ≤ 0.05 (1258 compounds) Max fold change ≥ 2 (450 compounds) 	Show compounds that have at least one of these tags: Hide compounds that have any of these tags:
<u>C</u> lear the filter	OK Cancel

To do this click on **Filter compounds**... and drag the new Tag on to the **Show** panel and click OK.

The table now shows the only the compounds that are separated by drift time. Click on **Separate by drift time** to view the detected compound ions with the same m/z and RT.

Aview Compounds Jaing this screen, you can find the compounds of the term or support of term	File Review Experiment Alignment Design Setup Peak Picking	Review Identify Deconvolution Compounds	Review Compound	Compou Is Statistic								nonlin A Waters C	omp		
Interset in your experiment. Neutral mass m/z z Retention time Drift time Peak Width Tag Accepted ID Identifications Anova (p) Max fold change 0 382,899.1797m/z cunknown> 899.1797 1 3.82 8.10 0.12 0 0.834 102 0 382,899.1797m/z cunknown> 899.1787 1 3.82 8.10 0.12 0 0.834 102 0 382,899.1797m/z cunknown> 899.1787 1 3.82 8.10 0.12 0 0.844 102 0 382,991.180m/z cunknown> 901.180 1 3.82 5.13 0.11 0 0 0.669 106 0 382,901.180m/z cunknown> 905.1792 //z 3.84 5.13 0.11 0 0 0.233 1.2 0 384.59500m/z cunknown> 905.1792 //z 2.84 3.67 0.13 0 0 0.233 1.2 0 384.59500m/z cunknown> 905.1792 //z 2.844 3.67 0.13 0	Review Compounds	Find a compound: Sear	ch	Q.	Filter com	npounds	 Filter is a 	tive) He		
In the table, sort and filter the compounds based on their resumments, to generate a shortlict. • How are the measurements, to generate a shortlict. • How are the measurements, calculated? To sort the table by a given value, gimply cide. Pier externation column header.		Compound	Neutral mass	m/z z	Retention time	Drift time	Peak Width	Tag 🔹	Accepted ID	Identifications	Anova (p)	Max fold cha	nge		
on their measurements, to generate a shortlist for further review. 0 algo selected composed a stage split selected composed and split selected compound a split selected compound the relevant selected compound a split selected compound and split selected compound a split selected compound and split selected compound a split selected compound and split selected compound a split selected compound the relevant selected compound a split selected compound and split selected compound a split selected compound a split selected compound and split selected compound a split selected compound a split selected compound and split selected compound a split sele	Create a shortlist to review	O 3.82_899.1797m/z	<unknown></unknown>	899.1797 1	3.82	8.10	0.12	-		0	0.894	1.02			
for further review.000 <th 0<="" colspan="2" td="" th<=""><td>In the table, sort and filter the compounds based</td><td>O 3.82_899.1867m/z</td><td><unknown></unknown></td><td>899.1867</td><td>3.82</td><td>5.08</td><td>0.03</td><td>4</td><td></td><td>0</td><td>0.421</td><td>1.73</td><td></td></th>	<td>In the table, sort and filter the compounds based</td> <td>O 3.82_899.1867m/z</td> <td><unknown></unknown></td> <td>899.1867</td> <td>3.82</td> <td>5.08</td> <td>0.03</td> <td>4</td> <td></td> <td>0</td> <td>0.421</td> <td>1.73</td> <td></td>		In the table, sort and filter the compounds based	O 3.82_899.1867m/z	<unknown></unknown>	899.1867	3.82	5.08	0.03	4		0	0.421	1.73	
To sort the table by a given value, simply click the relevant column header. Review the compound of interest, inspect the ions' algoment and peak picking: Review selected compound algoment and peak picking: Review selected compound algoment and peak picking: Review selected compound You can also double-click to review a compound You can also double-click to review a compound Second compound select one of its possible identifications as the accepted one. To speed this up, you can automatically accept identifications has: Score ≥ 4.80 Coccept identifications By exporting your data to betternal tools, there's nimit to your analysis.		O 3.82_901.1806m/z	<unknown></unknown>	901.1806	3.82	8.10	0.13	4		0	0.609	1.06			
the relevant column header. Review the compound's alignment and peak picking: Review selected compound alignment and peak picking: Review selected compound. Choose the correct identifications For each compound, select one of its possible identifications as the accepted one. To speed this up, you can automatically accept identifications Score ≥ 48.0 Review selected low. Compound 3.82_901.1809m/z Compound 3.82_901.1809m/z Compound 3.82_901.1809m/z Compound automatically accept identifications Score ≥ 48.0 Review selected low. Compound automatically accept identifications Review selected low.	How are the measurements calculated?	• 3.82_901.1809m/z	<unknown></unknown>	901.1809	3.82	5.13	0.11	-		0	0.0979	1.07			
Review the compounds alignment and peak pickings 0 3.84_d55.0920m/z 3.84 2.66 0.10 0 0.59 1.2 Review selected compound alignment and peak pickings Review selected compound as data select one of its possible identifications is the accepted one. 0 0.458 1.04 You can also double-click to review a compound, identifications as the accepted one. 0 0.458 1.04 Choose the correct identifications For each compound, select on of its possible identifications has: Score 2 4.8.0 Accept identifications the possible identifications by exporting your data to betwant loals, there's no limit to your analysis. Accept identifications alignment to your analysis. Image: mail to your analysis.		O 3.82_905.1792m/z	<unknown></unknown>	905.1792	3.82	5.13	0.08	-		0	0.233	1.2			
For each compound of interest, inspect the ions' alignment and peak pickings 0 0.438 104 Review selected compound Review selected compound 0 0.438 104 You can also double-click to review a compound, select one of its possible identifications So of the select one of its possible identifications is the accepted one. 0 0.438 104 Compound 3.82_991.1399/P/2 Compound 3.82_991.1399/P/2 Compound 3.82_991.1399/P/2 0 0.438 104 Choose the correct identifications Compound 3.82_991.1399/P/2 Compound 3.82_901.1809/P/2 Compound 3.82_901.1809/P/2 Compound 3.82_901.199/P/2 Compound 3.82_901.199/P/2 Compound 3.82_901.199/P/2 Compound 3.82_901.199/P/2 Compound 3.82_901.1809/P/2 Compound 3.82_901.199/P/2 Compound 3.82_90	the relevant column header.	O 3.84_455.0920m/z	<unknown></unknown>	455.0920	3.84	2.86	0.10	4		0	0.59	1.2			
alignment and peak picking: Review selected compound You can also double-click to review a compound. You can also double-click to review a compound. You can also double-click to review a compound. For each compound, select one of its possible To speed this up, you can automatically accept identifications in compounds where only one of the possible identifications Score $\ge 4.8.0$ Repet data for further processing By exporting your data to external tools, there's no limit to your analysis.		0 3.84_594.1359m/z	<unknown></unknown>	594.1359	3.84	3.67	0.13			0	0.458	1.04			
	For each compound, select one of its possible identifications as the accepted one. To speed this up, you can <i>automatically</i> accept identifications in compounds where only one of the possible dentifications have: Score ≥ 48.0 Accept identifications Export data for further processing By exporting your data to external tools, there's no limit to your analysis.		C_Norm			Drift time	A_LD		a/z vs. retent	ion time	-	m/z			

Waters

Exporting compound data

Compound data can be exported in a csv file format. You can either export the **compound measurements** or the **compound identifications.** As an example of data export, first clear any existing filters and then order the table on **Description** and highlight all the compounds which have a description.

File Review Experiment import Data Alignment Design Setup Peak Pickin	Review Deconvolutio	Identify on Compound	Review s Compounds	Compo Statist						- 1	nonline	-		
)						A Waters Comp	anı		
Review Compounds	Find a cor	mpound: Sea	rch	P	Filter com	pounds 💌					@ He	elp		
Jsing this screen, you can find the compounds of nterest in your experiment.	Tag 💌					Highest mean	Lowest mean	Isotope distribution	Max Abundance	Min CV%	Description			
Create a shortlist to review	(14709000	p-value ≤ 0.05	- 1 15-1	6 6.6E+03	B_HD	C_Norm	_ _	273.9158	1.67	Acetanilide	4		
In the table, sort and <u>filter the compounds</u> bas on their measurements, to generate a shortlist	d 🌗				Max fold change ≥ 2		6 1.5E+05	B_HD	A_LD				Alprazolam	
for further review.	- 4		entified	-1		B_HD			6182.8010		Caffeine			
How are the measurements calculate	2	-	entined ited by drift time	-1	6 Infinity	B_HD		 _	8736.4346			L		
To sort the table by a given value, simply click the relevant column header.	4	· · ·		-1	6 65	B_HD	C_Norm		1292.9505		Paracetamol	L		
the relevant column header.	4	New t	-	-1	6 6.52E+04	B_HD	C_Norm		12167.2199	1.85	Phenacetin	L		
Review the compounds	۹	Quick	Tags	-1	6 815	B_HD	C_Norm		11775.9523	4.29	Ranitidine	L		
For each compound of interest, inspect the ion: alignment and peak picking:	4	😭 Edit ta	gs	-1	6 3.34E+03	B_HD	C_Norm	 _	1168.2587		Tolbutamide	L		
Review selected compoun	4	Add to	Clip Gallery	16		B_HD	A_LD		18528.2297		Warfarin	L		
You can also double-click to review a compoun			0	0.331	1.24	C_Norm	B_HD		8.7816	17.46		١.		
the car also acapte there to review a compound	4				-	1				1				

Then right click on the highlighted compounds and create a New tag... called 'Confirmed Compounds'

Create new tag		e	
Confirmed Compounds			
	ОК	Cancel	

Click on Filter Compounds then drag the new tag onto the Show panel of the **Filter compounds** dialog and click **OK**.

Create a filter Show or hide compounds based on a selectio boxes to create the filter. For more guidance,	n of their tags. Move tags to the appropriate , please see the <u>online reference</u> .
Available tags: Not identified (5082 compounds) Anova p-value ≤ 0.05 (1258 compounds) Max fold change ≥ 2 (450 compounds) Separated by drift time (155 compounds)	Show compounds that have at least one of

Click on the File menu and select **Export compound identifications**, adjust the properties to be included in the export and click OK.

When you have saved the file a dialog opens allowing you to open the file if required:

QI Export	t Compound Identifications
	Export complete
	Open File Open Folder Close

Export Compound Identifications	х
Choose properties to be included in exported file	
Compound	
Compound ID	
Accepted?	
Adducts	
V Fomula	
Score	
Fragmentation Score	
Mass Error (ppm)	
✓ Isotope Similarity	
Theoretical Isotope Distribution	
Link	
Description	
Neutral mass (Da)	
m/z	
Charge	
Retention time (min)	
Anova (p)	
Max Fold Change	
Tags	
OK Cancel	

Exporting Compound Data to Pathways Tool(s)

Using Progenesis, you can export Compound lists to pathway analysis tools to help you understand your data in a wider biological context.

Currently Progenesis QI supports the export to:

IMPaLA: which aggregates and queries many other pathway analysis tools including KEGG and Reactome)

Export data for further processing

no limit to your analysis.

By exporting your data to external tools, there's

Export to pathways tool

IPA (Ingenuity Pathway Analysis): is a commercial web-based software application for the analysis, integration and interpretation of data derived from any experiments that generate gene and chemical lists with expression data, such as metabolomics and proteomics.

Support for the export of compound identity and expression data to either pathways tool is provided as standard.

Note: the use of IPA requires a local licensed copy, for licensing details please visit the IPA website.

The following section describes an example of the processes involved in exporting to and using **IMPaLA**. For additional details on the export of data to IPA refer to Appendix 8 (page 105)

Using IMPaLA

Typically, only accepted identifications are exported to pathway analysis tools. This is the default and recommended option.

First select the compound data to export to the pathways tool using tag filtering to 'focus' the set to export. For this example you can use the '**Confirmed Compounds'** set described in the previous section.

To ensure that the 9 Confirmed Compounds are the only ones displayed in the table set up the filter as shown below:

🛛 Progenesis QI Tutorial HDMSe - Progenesis QI						[
File Review Experiment		Review Compound				n	onlinea
Import Data Alignment Design Setup Peak Picking I	Deconvolution Compounds Col	mpounds Statistics				A	Waters Compar
Review Compounds	Find a compound: Search	P	Filter compounds	 Filter is a 	active		🕜 Help
Using this screen, you can find the compounds of interest in your experiment.	Compound Neutra	al mass m/z z	Rete Filter compound	ds	īag ▼	Accepted ID	Identificatic
Create a shortlist to review	• 4.42_136.0758m/z <unkn< td=""><td>iown> 136.0758 1</td><td>4.42</td><td></td><td>ک</td><td>14708992</td><td>1</td></unkn<>	iown> 136.0758 1	4.42		ک	14708992	1
In the table, sort and filter the compounds based on their measurements, to general as an		200,0020 1	Clear filter			7847292	1
for further review.	npounds		×	0.16	٨	46511425	2
How are the measurem Create a fil				0.18	٨	855682	1
	compounds based on a selection e the filter. For more guidance,			0.15		46506142	4
				0.18		49854487	4
2 Review the compounds For each compound of interest, in Available tags:		Show compounds t tags:	hat have all of these	0.27	4	7847488	1
	fied (5082 compounds)		pounds (9 compounds)	0.14	4	9357	1
	value ≤ 0.05 (1258 compounds)	Commed com	pounds (9 compounds)	0.18	٨	4702	1
You can also double-click to revie	change ≥ 2 (450 compounds) by drift time (155 compounds)	Show compounds t these tags:	hat have at least one of				,
2 Choose the correct identificati				tage			
For each compound, select one of identifications as the accepted on				age			_
To speed this up, you can <i>autome</i> identifications in compounds when the possible identifications has:		Hide compounds th tags:	at have any of these			B_HD	
Score ≥ 48.0 Accept						₽ ● E	
4 Export data for further proces By exporting your data to externa no limit to your analysis.			OK Cancel				
Export to pathways tool	Arcsinh Normalis						
	₹ 0.10 — - -0.95 —	1 4 1					
Experiment design	•						÷
Review your data from a different perspective: Current design: Minus QC						Section C	omplete 🤆

Then click Export to pathways tool, and select IMPaLA from the Pathways tools and click Next.

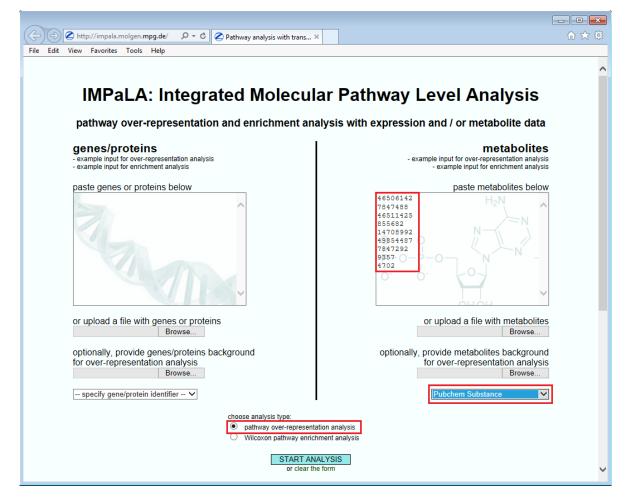
Ensure that Accepted IDs only is selected.

Select either Pathway over-representation analysis or Wilcoxin pathway enrichment test.

Make sure the **Open IMPaLA in my browser** is ticked and then click **Copy compounds to clipboard.**

QI Export Pathways Information	×
Configure your export Choose which identifications to export and the type of analysis you want to perform.	
Select which identifications to export:	
Accepted IDs only (recommended) All possible IDs	
Select the type of analysis to perform:	
Pathway over-representation analysis	•
Pathway over-representation analysis	
Wilcoxon pathway enrichment analysis	
Open IMPaLA in my browser	
< Back Copy compounds to clipboard Close	e

When IMPaLA opens, paste the metabolites in to the right hand panel.



Select specific metabolite identifier

Click Start Analysis

Analysis results list is returned:

 metabolite background size is 4612. 32 pathways found. 				Results per page: 50 V Go to page (previous) 1 of 1 (next)		
pathway name	pathway source	overlapping metabolites	all metabolites	P _{metabolites}	Q _{metabolites}	
Warfarin Pathway, Pharmacodynamics	PharmGKB	1	1 (1)	0.000867	1	
Warfarin Pathway, Pharmacokinetics	PharmGKB	1	1 (1)	0.000867	1	
Warfarin Action Pathway	SMPDB	1	5 (5)	0.00433	1	
Benzodiazepine Pathway, Pharmacokinetics	PharmGKB	1	11 (11)	0.00951	1	
Xenobiotics	Reactome	1	51 (51)	0.0435	1	
Cytochrome P450 - arranged by substrate type	Reactome	1	115 (116)	0.0961	1	
Phase 1 - Functionalization of compounds	Wikipathways	1	137 (137)	0.114	1	
Phase 1 - Functionalization of compounds	Reactome	1	150 (151)	0.124	1	
Defective CYP27A1 causes Cerebrotendinous xanthomatosis (CTX)	Reactome	1	150 (151)	0.124	1	
Defective CYP1B1 causes Glaucoma	Reactome	1	150 (151)	0.124	1	
Defective CYP4F22 causes lchthyosis, congenital, autosomal recessive 5 (ARCI5)	Reactome	1	150 (151)	0.124	1	
Defective CYP11B2 causes Corticosterone methyloxidase 1 deficiency (CMO-1 deficiency)	Reactome	1	150 (151)	0.124	1	
Defective CYP24A1 causes Hypercalcemia, infantile (HCAI)	Reactome	1	150 (151)	0.124	1	
Defective MAOA causes Brunner syndrome (BRUNS)	Reactome	1	150 (151)	0.124	1	

Click to explore returned analysis.

Note: the success of a Pathways analysis is dependent on the organism under study being available to search using **IMPaLA**. This data set is not truly a suitable example but returns results that at least demonstrate the process.

Now move to the Compound Statistics section by clicking section complete.

Stage 10: Compound Statistics

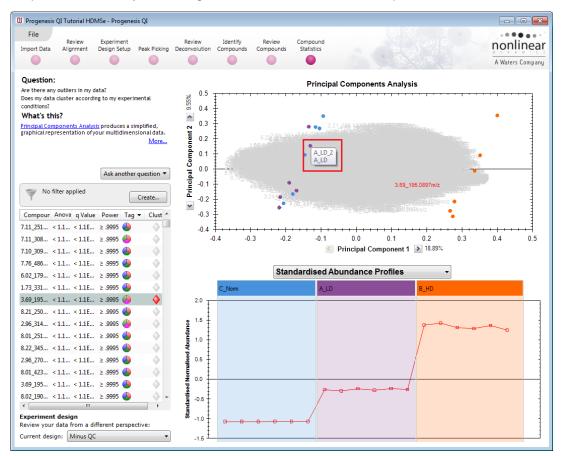
The user guide now describes the functionality of the Multivariate Statistics.

Compound Statistics opens calculating the Principal Components Analysis (PCA) for the active 'tag' filter if one exists.

Note: make sure you have cleared any Tag Filters before performing Principal Components Analysis (PCA)



As an example we will start by examining the PCA for all of the 5152 compounds.



The statistical analysis of the data is presented to you in the form of interactive graphical representation of answers to questions asked of the analysed data.

Note: the runs (samples) are displayed as solid coloured circles on the plot. To identify the runs, a tooltip is displayed when the cursor is held over each circle.

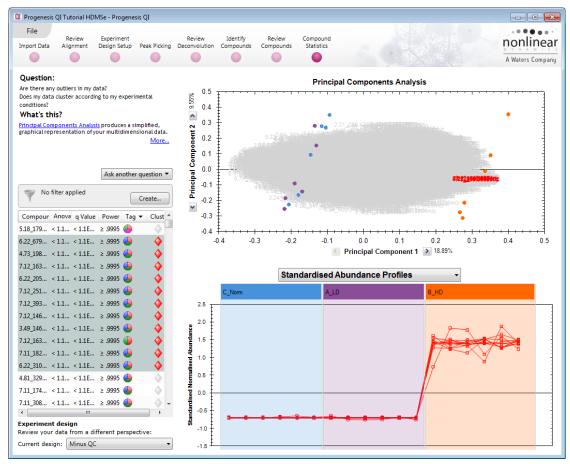
Principal Component Analysis (PCA)

In **Compound Statistics** the first statistically based question asked of the data takes the form of a Quality Control assessment:

Are there any outliers in my data? And does my data cluster according to my experimental conditions?

It answers this question by: 'Using Principal Components Analysis (PCA) to produce a simplified graphical representation of your multidimensional data'.

PCA can be used to determine whether there are any outliers in the data and also look at how well the samples group. The groupings that can be observed on the 2D PCA plot can be compared to your experimental conditions and conclusions can be drawn regarding possible outliers in your data. Selecting compounds in the table will highlight the compounds on the 'Biplot' and their abundance profiles will appear in the lower panel.



Note: the table in the Statistics view contains additional columns:

q value: tells us the expected proportion of false positives if that feature's p-value is chosen as the significance threshold

Power: can be defined as the probability of finding a real difference if it exists. 80% or 0.8 is considered an acceptable value for power. The Power Analysis is performed independently for each compound, using the abundance variance, sample size and difference between the means.

Also, for a given power of 80% we can determine how many samples are required to ensure we find a difference if it actually exists.

Note: Power analysis is discussed in Appendix 9 (page 108)

Correlation Analysis

Use the tags created in Review Compounds to filter the compounds displayed in the table. We are going to explore the Correlation Analysis for all the Compounds (with possible identifications) that display a significant 2 fold or greater difference in abundance.



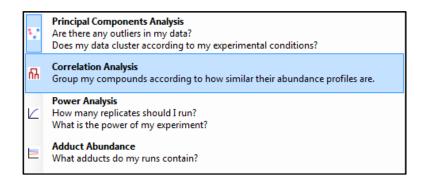
To filter the data click Create

Set up the filter as shown below

QI Filter the compounds Create a filter Show or hide compounds based on a selection boxes to create the filter. For more guidance,	
Available tags: Not identified (5082 compounds) Anova p-value ≤ 0.05 (1258 compounds) Separated by drift time (155 compounds) Confirmed Compounds (9 compounds)	Show compounds that have all of these tags: Max fold change ≥ 2 (450 compounds) Show compounds that have at least one of these tags: Hide compounds that have any of these tags:
Clear the filter	OK Cancel

On pressing OK the PCA will recalculate.

To set up the **Correlation Analysis** using this filtered data set click on **Ask another question** (above the table). A selection of 4 tools will appear in the form of questions



Select the second option to explore 'feature correlation based on similarity of abundance profiles'



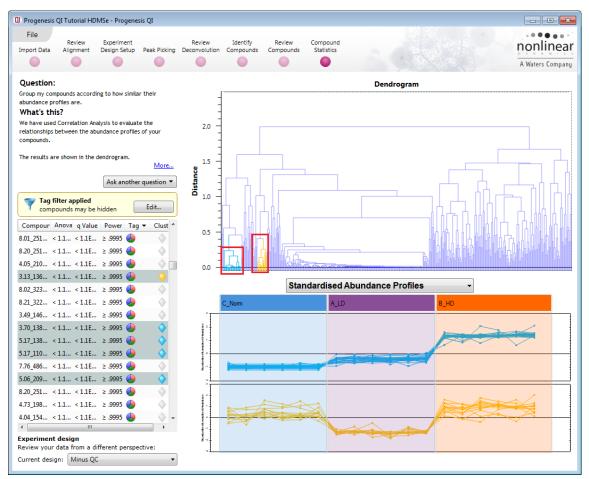
This time the statistically based question(s) being asked is:

'Group my (selected) compounds according to how similar their expression profiles are'

The question is answered by:

'Using Correlation analysis to evaluate the relationships between the (selected) compounds' expression profiles'.

The answer is displayed graphically in the form of an interactive dendrogram where the vertical distance, between each branch can be taken as indicative of how similar the abundance profiles of each cluster of compounds are to each other.



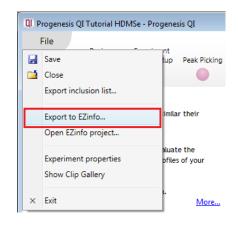
Correlation Analysis enables the grouping of compounds together according to how similar their abundance profiles are.

Clicking on a branch on the Dendrogram selects the compounds on the table. You can then tag this group, of 'potentially' related compounds, by right clicking and creating a 'New tag' for them. Then use the tag to focus on them at the **Review Compounds** stage.

Exporting to EZinfo

To export data to EZinfo select the option from the file menu.

Note: EZinfo only appears in the Progenesis QI File menu if it has been installed. Therefore, this section is only relevant to users who have access to EZinfo.



For this example, before exporting to EZinfo, please ensure that you have created an Experimental Design Setup to compare the A_LD and B_HD conditions. To do this, return to Experiment Design Setup set up and create an additional design for 'Low vs High' as shown below.

OI Progenesis QI Tutorial HDI File Import Data Alignment	Experiment Design Setup Peak Picking	Review Identify Review Deconvolution Compounds Compounds	Compound Statistics	Maximize nonlinear A Waters Company
All conditions	Minus QC Lo	ow vs High I 🗙 🗋 New		Ø Help ▼
Setup conditions		Runs Add Selected Runs to Condition	▼ Search ۶	
Setup the conditions that y (e.g., control, drug A, etc), your samples to the correc	, and then assign each of	C_Norm_1	C_Norm_2	C_Norm_3
Low	Delete		and the second	Sec.
	A_LD_1 Remove			
	A_LD_2 <u>Remove</u> A_LD_3 <u>Remove</u>			
	A_LD_4 Remove	C_Norm_4	C_Norm_5	C_Norm_6
	A_LD_5 Remove	E. C.	Sec.	E. C.
	A_LD_6 Remove			
📮 High	Delete			
	B_HD_1 Remove	D_QC_1	D_QC_2	D_QC_3
	B_HD_2 Remove		Sale .	2240
	B_HD_3 Remove			
	B_HD_4 Remove			
	B_HD_5 <u>Remove</u> B_HD_6 <u>Remove</u>	D_QC_4		
Add condition				Section Complete
				Section Complete 🤿

Return to **Compound Statistics**, make sure that there is no Filter applied, then select **Export to EZinfo**. You will be asked to save the project before EZinfo opens.

Q Progenesis QI Tutorial HDMSe - Progenesis QI					
File					
Save tup Peak P	Review Identify cking Deconvolution Compounds	Review Compound Compounds Statistics			nonlinear
Close					A Waters Company
Export inclusion list					
Export to EZinfo	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Pri	ncipal Components An	alysis	
Open EZinfo project	QI Save As			×	
	🔾 🗸 🖓 🗸 Prog 🕨	Progenesis QI Tutorial 🕨	✓ ⁴ → Search Pr	rogenesis QI Tutorial 🔎	
Experiment properties s a simplified imensional of the second sec		er		!≡ ▾ 🔞	
Show Clip Gallery	ore. Downloads	▲ Na	me	Date A	
× Exit	😌 Dropbox	=	A_LD_1.raw	28/1(= 2.0388m	
	🔛 Recent Places		A_LD_2.raw	28/10 2.0388m 28/10	1/2
Ask another question	n ShareFile BitTorrent Sync		A_LD_3.raw	28/1(
No filter applied			A_LD_4.raw	28/1(
Create			A_LD_5.raw A_LD_6.raw	28/1(0.4 0.5
	ust 🗦 🕞 Libraries		B_HD_1.raw	28/1(0.4 0.5
-	Documents	+ + _		•	
7.12_163 < 1.1 < 1.1E ≥ .9995	File name: Prog	enesis QI Tutorial HDMSe.usp			
	Save as type: UMet	rics EZinfo project (*.usp)			
	Save as type.				
-				-	
	Hide Folders		Save	Cancel	
4.05_210 < 1.1 < 1.1E ≥ .9995	- Hide Folders		2010		
7.46_214 < 1.1 < 1.1E ≥ .9995	Ē 0.0		/		
5 02 202 211 211E > 0005	tuning of the second se				
Experiment design					
Review your data from a different perspective:	-1.0 D		•d		
Current design: Low vs High	-1.5				

Save the EZinfo project in the same folder as your Progenesis QI experiment.

A dialog for **Collecting data from EZinfo** opens in Progenesis QI and EZinfo opens displaying the exported data.

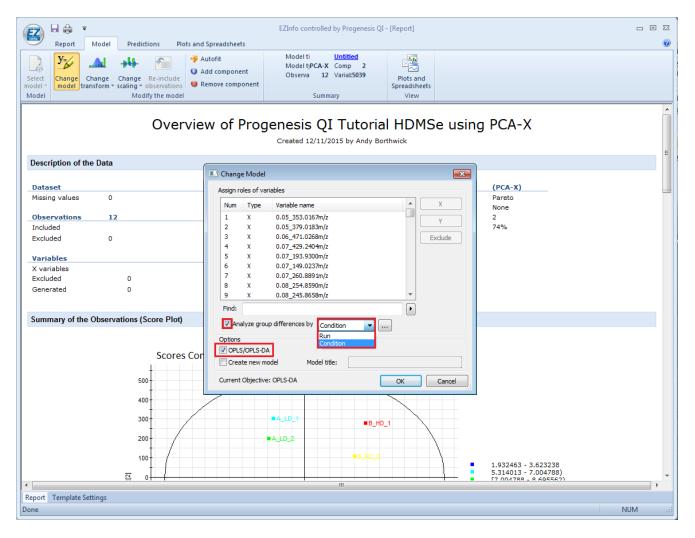
Note: the dialog may be obscured by the EZinfo application as it opens.

Ensure that Pareto Scaling is currently selected, if it is not current, then use **Change Scaling** in the 'Model tab' to set it to Pareto.

As we have specifically exported data to EZinfo for the comparison of 2 conditions we can generate an S-plot from a discriminate analysis (OPLS).

Select the compounds you are interested in on the EZinfo Loadings plot, 'Transfer Loadings data' link to return a batch of compounds to Progene							
'Transfer Loadings data' link to return a batch of compounds to Progene							
The compound batch will appear below where you can tag all the compo for identification or further investigation.	The compound batch will appear below where you can tag all the compounds in it ready for identification or further investigation.						
Right-click on a batch to assign a tag to them.							
Batch Compounds (count) Tag 💌							
Import tags	Cancel						

To do this in EZinfo with the exported data, first change the model of the data by selecting Change Model

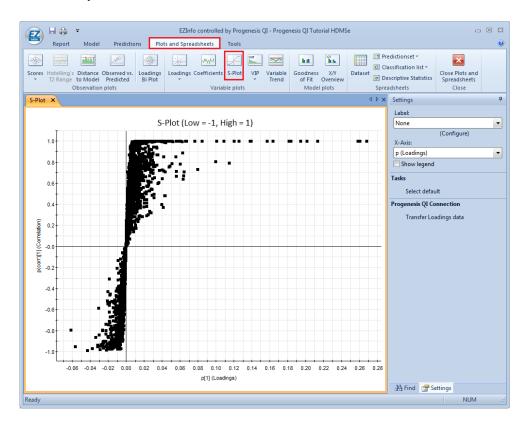


Select analyse group differences by Condition.

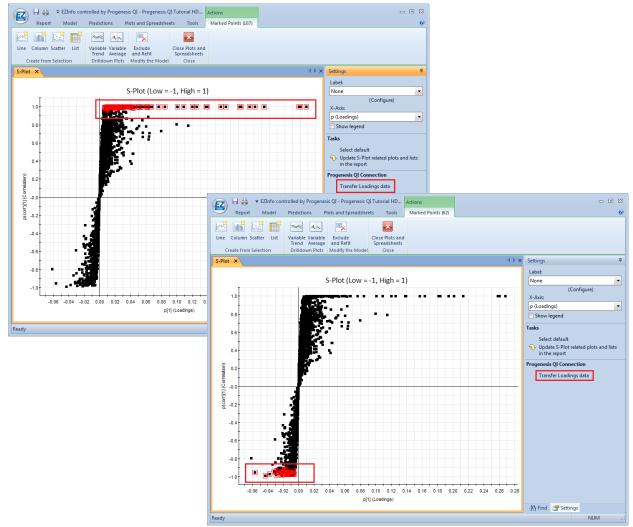
Set the options to OPLS/OPS-DA and click OK

The PCA will update the new model.

Now Select Plots and Spreadsheets on the main tool bar and click on the S-Plot



Select a set of points and click on **Transfer Loadings data**. Repeat this process for other sets of data.



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As you transfer each group of selected data from the loadings plot a new batch appears in the **Collecting data from EZinfo** dialog.

Clear the filter

Select and right click on each batch in turn and create a **New tag...**, naming it appropriately.

Create new tag	×
S_Plot_A	
	OK Cancel

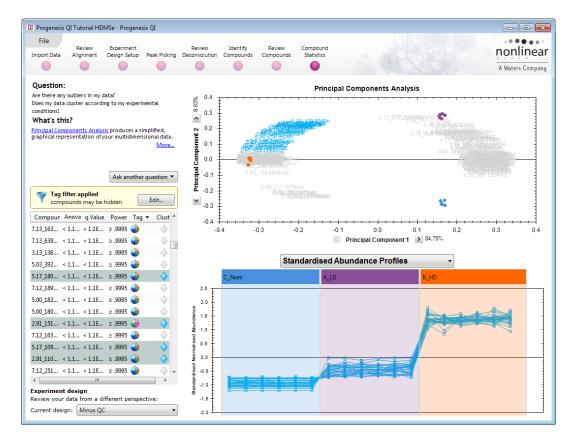
Q Collecting data from EZinfo	_ • •				
Select the compounds you are interested in on the EZinfo Loadings plot, then click the 'Transfer Loadings data' link to return a batch of compounds to Progenesis QI.					
The compound batch will appear below where you can for identification or further investigation.	tag all the compounds in it ready				
Right-click on a batch to assign a tag to them.					
Batch Compounds (count) Tag 🔻					
1 187 🕒					
2 85 🥥					
	Import tags Cancel				

Click Import tags

Close the dialog and EZinfo. The tags will now be available in to use in Progenesis QI.

	u) Pilter the compounds			
	Create a filter Show or hide compounds based on a selection of their tags. Move tags to the appropriate boxes to create the filter. For more guidance, please see the <u>online reference</u> .			
Set a new Tag Filter in Compound Statistics to Show compounds that have at least one of these tags.	Available tags: Not identified (5082 compounds) Anova p-value ≤ 0.05 (1258 compounds) Max fold change ≥ 2 (450 compounds) Separated by drift time (155 compounds) Confirmed Compounds (9 compounds)	Show compounds that have all of these tags: Show compounds that have at least one of these tags: Splot_A (187 compounds) Splot_B (85 compounds) Hide compounds that have any of these tags:		

Click OK to create the new PCA based on these tags.



Note: as you close Progenesis QI, EZinfo will also close.

Cancel

OK

Appendix 1a: Stage 1 Data Import and QC review of data set

You can use your own data files, either by directly loading the raw files (Waters, Thermo, Bruker, Agilent and ABSCiex) or, for other Vendors, convert them to mzML or mzXML format first.

To create a new experiment with your files select **New** give your experiment a name and then select a location to store the experiment files. Click **Next**.

Q Progenesis QI			
File			
Experiments			nonlinear
			A Waters Company
Recent experiment	Search	٩	Getting started Here are some resources to help you get started with Progenesis QI: • Fundamental concepts of the Progenesis QI workflow
	Q Create New Experiment	×	User guide and tutorial data
	Create a new ESI experiment Analyse samples run using electrospray injection (ESI) to generate the charge on compounds.		EZinfo version information
	Enter a name for the new experiment:		Version 3.0.3.0 of EZinfo is installed.
	ProgenesisQI_Tutorial HDMSe		
	Select the location to store the experiment files:		Quickly go to an ion map location
There	Save experiment in the same folder as the run data	nts	Want to quickly validate your sample running by zooming to a known ion?
	Back Next Canc	el	8
			Jump to a specific m/z and RT using the <u>Go To Location tool</u> in the top-left corner of the ion maps.
			Latest blog posts
Other experiments			rew Food for thought at RAFA 2015 Big society for small molecules
🎦 New 🛛 🚰 Op	en	2.2.5794.50409	HUPO 2015: Proteomics, Progenesis and poutine

Now select the machine type that was used to run the samples.

QI Create New Experiment	
Set the analysis parameters The type of machine, and the settings used, determine analysed.	e how your runs must be
What type of machine was used to run the samples?	
High resolution mass spectrometer	
Select the runs' data format: O Profile data Centroided data Resolution (full width at half maximum): 50000	High resolution mass spectrometer e.g. Waters SYNAPT G2/G2-S, AB SCIEX TripleTOF, Agilent QTOF, Bruker Maxis, Thermo LTQ Orbitra Thermo LTQ Iontrap in Enhanced mode. Low resolution ion trap
Select the ionisation polarity for this analysis:	e.g. Bruker HCT, Bruker HCT Ultra, Thermo LTQ XL Thermo FT-ICR
Positive	
Negative	Direct sample analysis Pre-processed data from Progenesis Bridge
Back	Next Cancel

Note: if you have converted or captured the data as centroided then select Centroided data and enter the Resolution (full width at half maximum) for the MS machine used.

In this example the data was captured in **Profile** mode.

QI Create New Experiment	×
Set the analysis parameters The type of machine, and the settings used, determine how your runs must be analysed.	
What type of machine was used to run the samples?	
High resolution mass spectrometer 🔹	
Select the runs' data format: Profile data Centroided data Resolution (full width at half maximum): 50000 Select the ionisation polarity for this analysis: Positive 	
Negative Back Next Cance	:el

Finally select the ionisation polarity, either **positive** or **negative**, this will determine which list of possible adducts is available on the next page.

0 Create New Experiment	0 Create New Experiment
Select the possible adducts To correctly identify the compounds in your samples, we need to know how the ions are formed.	Select the possible adducts To correctly identify the compounds in your samples, we need to know how the ions are formed.
Adducts in library: Adducts in this experiment: C M+H-2H2O >> C M+H-H2O >> C M+H-H2O < M+H M+H M+H M+H M+H M+H M+H M+H M+H M+CH3OH+H M+CH3OH+H M+ACN+H M+2H M+H+Na	Adducts in library: M+Cl M+K-2H M+K-2H M-2H M-3H 2M+FA-H 2M+FA-H M-3H E Move adducts into this list to add them to your experiment. Edit adduct library
Back Create experiment Cancel	Back Create experiment Cancel

To add an adduct to the library select **Edit adduct library...** and then click **Add**. Now provide the Mass (monomer, dimer or trimer), Adduct, Adduct Mass and Charge of the new adduct and then add it to the library.

QI Adduct Library				- • •		Q Create Ne	w Adduct		X
The adducts listed to experiments. Show: All Po				hen creating new	×	Enter the n Masses:	ame and deta Adduct:	ails of the new ad Adduct Mass:	duct. Charge:
Adduct	Adduct Mass	Masses	Charge	<u>A</u> dd		2M 🔹	+K	38.9632	1 日
🚹 M+H	1.0073	1	1	Remove					
🔁 M+NH4	18.0338	1	1				ſ		Connel
🔁 M+Na	22.9892	1	1	E			l	Add	Cancel
🚼 M+CH3OH+H	33.0335	1	1						
🔁 М+К	38.9632	1	1						
🗄 M+ACN+H	42.0338	1	1						
🖶 M+2Na-H	44.9712	1	1	_					
			<u>S</u> ave	Cancel					

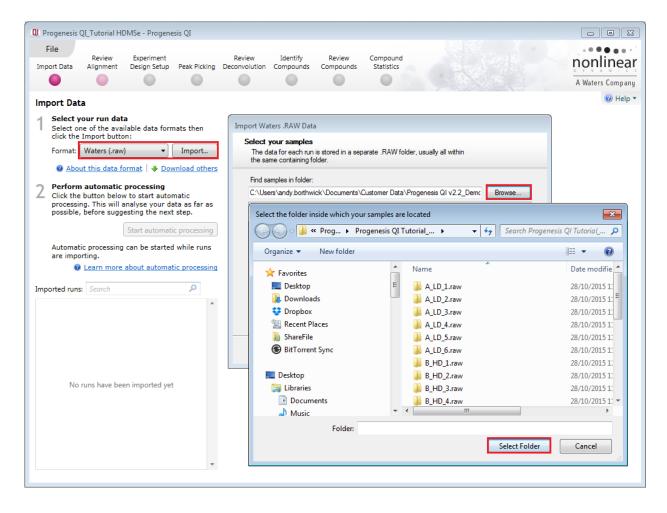
Now select the expected adducts based on you knowledge of your experimental conditions. In this example the polarity was positive and a basic set of Adducts was selected as shown on the right panel below.

dducts in library:			Adducts in this experimer	it:
🗄 M+2Na-H		>>	M+H-2H ₂ O	-
🗄 M+ACN+Na			■ M+H-H ₂ O	
🛨 M+2Na		<<	• M+H	
🗄 M+3H	=		H+NH4	
🗄 M+2H+Na			🖬 M+Na	
🗄 M+2Na+H			M+CH ₃ OH+H	
🗄 2M+H			М+К	
2M+NH4			M+ACN+H	
🗄 2M+Na			M+2H	
2M+3H ₂ O+2H	-		M+H+Na	

Click Create experiment to open the Import Data stage of the workflow.

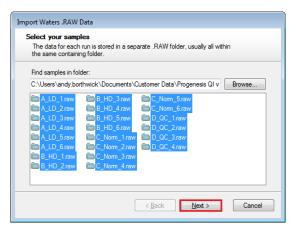
Select the 'Import Data file format', in this example they are Waters (.raw) files

Then locate your data files using Import...



Use Browse to locate and select the folder containing all the Data files, in this example (A_LD_1 to D_QC_4).

Select the required **.raw** folders for your experiment and click **Next.**



Import Waters .RAW Data			
Lock mass calibration All of your samples contain lock mass calibration information. Please provide the calibration m/z.			
Perform lock mass calibration			
Lock mass m/z: 556.2766			
Note: If you have already calibrated your data externally, you should not perform calibration here.			
< <u>B</u> ack <u>N</u> ext > Cancel			

Import Waters .RAW Data
Ready to import Please review the information below before starting the import process.
Your samples are ready to be imported. Please review the options below.
22 samples selected for import.
Lock mass calibration: Yes Lock mass m/z: 556.2766
< Back Import Cancel

The folders will be examined and the Dialog will ask you for Lock Mass Calibration details.

If this has already been performed externally then untick this option.

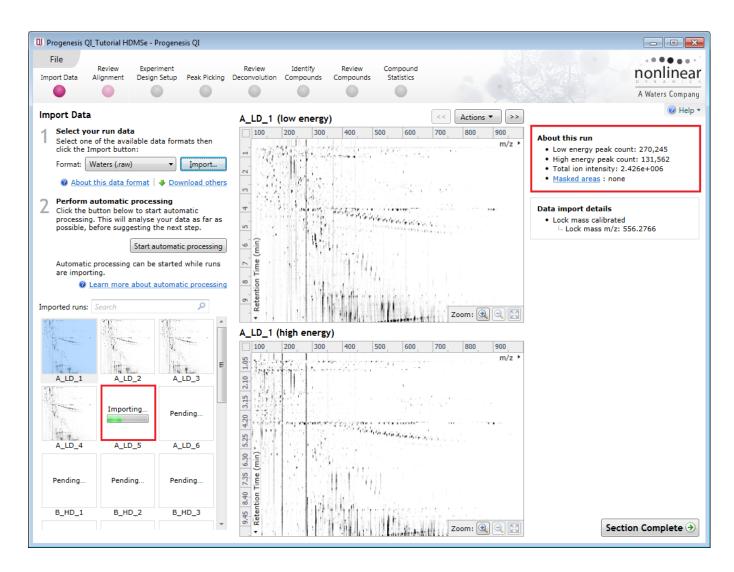
Note: for the Tutorial data Lock mass calibration is required.

Finally a summary page appears. Click Import

On loading the selected runs your data set will be automatically examined and the size of each file will be reduced by a 'data reduction routine', which reduces the data by several orders of magnitude but still retains all the relevant quantitation and positional information.

Note: For a large number of files this may take some time.

Each data file appears as a 2D representation of the run. At this stage, assuming you have selected 'profile data' (page 79) you will be warned if any of the data files have been 'centroided' during the data acquisition and conversion process.



Note: as each data file is loaded the progress is reported in the **Import Data** list. The dialog below the run reports on the QC of the imported Data files. In this case 'No problems found' with this data file.

Note: details of the current run appear on the top right of the view.

Note: as the loading process starts you can also **set up and start the Automatic Processing** before the loading has completed. This is a 4 stage process that involves the selection of an Alignment Reference (either automatically or manually), then the Automatic Alignment of all your runs to this Reference run followed by setting up of an Experiment Design and Peak Picking.

Click Start automatic processing to start the automatic alignment of your runs.

Appendix 1b: Stage 1 Data Import from UNIFI (Waters Only)

LCMS data that has been acquired fron Waters Vion and Xevo machines where the acquisition is managed through the UNIFI Scientific Information System, is exported **Uncompressed** from UNIFI as a Unifi Export Package (UEP) format file.

To create a new experiment in Progenesis QI, using a .UEP file, select **New** give your experiment a name (Progenesis UEP example) and then select a location to store the experiment files. Click **Next**.

🛿 Progenesis QI			
File Experiments			nonlinea A Waters Compan
Recent experimen	ts	Search P	Getting started Here are some resources to help you get started with Progenesis Q1: • Eundamental concepts of the
There	QI Create New Experiment Create a new ESI experiment Analyse samples run using electrospray injection (ESI) to generate the compounds. Enter a name for the new experiment: Progenesis UEP example Select the location to store the experiment files: Save experiment in the same folder as the run data Choose an experiment folder 	e charge on Browse	Progenesis Q1 workflow • User guide and tutorial data • Frequently-asked questions EZinfo version information Version 3.0.3.0 of EZinfo is installed. Quickly go to an ion map location Want to quickly validate your sample running by zooming to a known ion? 500 Go To Location
Other experiment:		Cancel	Sump to a specific m/z and RT using the Go To Location tool in the top-left corner of the ion maps. Latest blog posts 6 ways Progenesis OI can help with your compound identification Season's Greetings from all at Nonlinear!

Now select the machine type, polarity that was used to run the samples. Then select the expected adducts based on you knowledge of your experimental conditions.

QI Create New Experiment	Create New Experiment	—X —
Set the analysis parameters The type of machine, and the settings used, determine how your runs must be analysed.	Select the possible adducts To correctly identify the compounds in your samples, we need to know h are formed.	now the ions
What type of machine was used to run the samples? High resolution mass spectrometer Select the runs' data format: Profile data Centroided data Resolution (full width at half maximum): Select the ionisation polarity for this analysis: Positive Negative Negative	Adducts in library: Adducts in this experimental strength of the second strengt of the seco	iment:
Back Next Cancel	Back Create experiment	Cancel

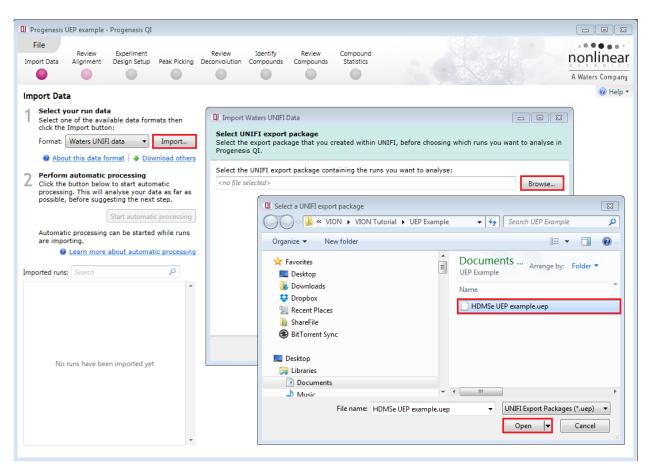
Click Create experiment to generate the experiment.

When Import data opens select **Waters UNIFI data** as the import data format.

Then click Import.

1	Select or	rour run data ne of the available data for Import button:	mats then
	Format:	Waters UNIFI data 🔹 🔻	Import
	@ <u>Abo</u>	Waters UNIFI data Version: 1.0.5795.29673	vnload others
2	Perforn Click the		matic
	processi possible	AB SCIEX (.wiff) Version: 1.0.5750.21225	ata as far as t step.
		Bruker Daltonics (.d) Version: 1.0.5673.24544	ic processing
	Automat	mzML Files Version: 1.0.5750.21209	d while runs
	are impo	mzXML files Version: 1.0.5750.21198	tic processing
Imported runs	Thermo (.raw) Version: 1.0.5799.22379	0	
	oncertuns	Thermo FT-ICR (.raw) Version: 1.0.5799.22379	A

Browse to the location of the UEP and open the file

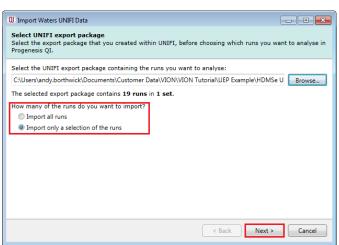


Depending on the data in UNIFI the exported package may contain 1 or more sample sets. In this example there is only one, containing 19 samples.

You have the option of importing all the samples or a selection.

In this example we will import a selection of the samples.

Click Next.



ou want to analyse from				
elect the samples you wa Sample name	ant to import: Ready to ID?	Fragmentation method	Ion mobility?	Replicate #
ample 1 [1]	Yes	MSE	Yes	1
ample 2 [1]		MS ^E		1
ample 3 [1]		MS ^E		1
ample 4 [1]	Yes	MS ^E	Yes	1
ample 5 [1]	Yes	MS ^E	Yes	1
ample 6 [1]		MS ^E		1
ample 7 [1]	Yes	MS ^E	Yes	1
		MS ^E		1
ample 9 [1]	Yes	MSE	Yes	1
ample 10 [1]	Yes	MSE	Yes	1
	W	MACE	M	1

Select the samples to import.

Click Import.

As importing from the UEP starts you can set up the **automatic processing** to run on the data as described in Section 3 of this guide (page 8).

QI Progenesis UEP example - Progenesis QI		
File Import Data Alignment Design Setup Peak Picking	Review Identify Review Compound Deconvolution Compounds Statistics	nonlinear A Waters Company
Import Data	Sample 1 [1] (low energy)	🕡 Help 🔻
Select your run data Select one of the available data formats then click the Import button: Format: Waters UNIFI data Waters UNIFI data Import About this data format & Download others	100 200 300 400 500 600 700 800 900 About t N Image: State of the state of	t his run v energy peak count: 347,874 h energy peak count: 246,544 al ion intensity: 2.327e+005 s <u>ked areas</u> : none
2 Perform automatic processing Click the button below to start automatic processing. This will analyse your data as far as possible, before suggesting the next step.	To compensate for units in recention time, an runs in the experiment must be aligned	nport details ss section area calibration exists.
Start automatic processing Automatic processing can be started while runs are importing. Learn more about automatic processing Imported runs: Search P Sample 1 [1] Sample 2 [1] Sample 3 [1]	How do you want to choose your alignment reference? Assess all runs in the experiment for suitability Use the most suitable run from candidates that I select Use this run: Sample 1 (1)	
I Valle I Philes I Parks	For information on choosing the alignment reference, and why you might want to select your own candidates, please see the <u>online guidance</u> .	
Sample 4 [1] Sample 5 [1] Sample 6 [1]	< Back Next > Cancel	
Sample 7 [1] Sample 8 [1]		Section Complete ④

Once the importing is complete, the Automatic processing of the data proceeds.

Q Progenesis UEP example - Progenesis QI					
File Import Data Alignment Design Setup Peak Picking	Review Identify Deconvolution Compound	Review s Compounds	Compound Statistics		nonlinear A Waters Company
Import Data	Sample 1 [1] (low	energy)		<< Actions >>	😢 Help 🔻
1 Select your run data 2 Select one of the available data formats then click the Import button: Format: Waters UNIFI data ● Import @ About this data format ♦ Download others 2 Perform automatic processing Click the button below to start automatic for a formation for a format.	□ 100 200 30 	0 400 50	0, 600,	700 800 900, m/z •	About this run • Low energy peak count: 347,874 • High energy peak count: 246,544 • Total ion intensity: 2.327e+005 • Masked areas : none
processing. This will analyse your data as far as possible, before suggesting the next step.					s run is being used as the experiment's nment reference.
C Restart automatic processing	Automatic process Current step: Analysing	-			ou want to use a different run as the nment reference, you'll need to discard
Automatic processing can be started while runs are importing.	 Importing runs: Selecting reference: Aligning runs: Creating design: Peak picking: 	8 of 8 processed Sample 1 [1] 7 of 7 processed Created Picking			analysis and restart the automatic cessing: Restart automatic processing a import details Cross section area calibration exists.
Sample 1 [1] Sample 2 [1] Sample 3 [1]				Cance	al
Sample 4 [1] Sample 5 [1] Sample 5 [1]	10 8 6 4 Actention Time (min)			La L	Section Complete 🔿

Once the analysis completes refer to page 12 in the guide.

Appendix 2: Stage 2 Processing warnings and failures

If a stage fails to complete successfully or only partially completes, the automatic processing dialog will warn you of the problem. This may or may not allow the automatic processing to complete.

For example, a run that fails to automatically align will trigger a warning, although analysis will continue; however, the automatic processing dialog will warn you that runs failed to align. On completion of the Automatic processing go to the **Review Alignment** stage to investigate the problem.

Time taken: 9 minutes 58 s	ng complete (with warnings).	
 Importing runs: 	22 of 22 processed	
 Selecting reference: 	D_QC_3	
Aligning runs:	21 of 21 processed	
	A 1 run failed to align - continuing without it	
 Creating design: 	Created	
 Peak picking: 	6069 compound ions found	

Note: in this example the run that failed to align will not contribute to the peak picking and will be excluded at the alignment stage (a cross appears in the include column).



You can either remove the run from the experiment at **Import Data** or add it back in at the **Review Alignment** stage once the alignment of the run has been corrected.

As another example, runs that import successfully but with warnings at the **Import Data** stage will cause a flag in the readout to notify you of the potential quality issue.

If some runs in a data set fail to import (but not all), the automatic processing will continue informing you that one or more runs have failed to import.

Q Processing Complete			×
Automatic process Time taken: 10 minutes 2	ing complete (with 21 seconds	warnings).	
A Importing runs:	22 of 22 processed 1 failed to import		
 Selecting reference: 	D_QC_3		
 Aligning runs: 	20 of 20 processed		
 Creating design: 	Created		
 Peak picking: 	6069 compound ions fo	ound	
			Close

In this case you can remove the runs at Import Data and if appropriate replace them with additional runs.

Note: adding additional runs will then be aligned and peak picking should be re-done to include data from the added runs in the generation of the aggregate.

An example of a problem that would halt the automatic processing would be the failure to successfully import all the potential reference candidates, (for example: while importing, you specified the selection of the alignment reference to be made from several runs before they were fully imported and set the processing underway, and they later failed to import owing to problems with the runs).

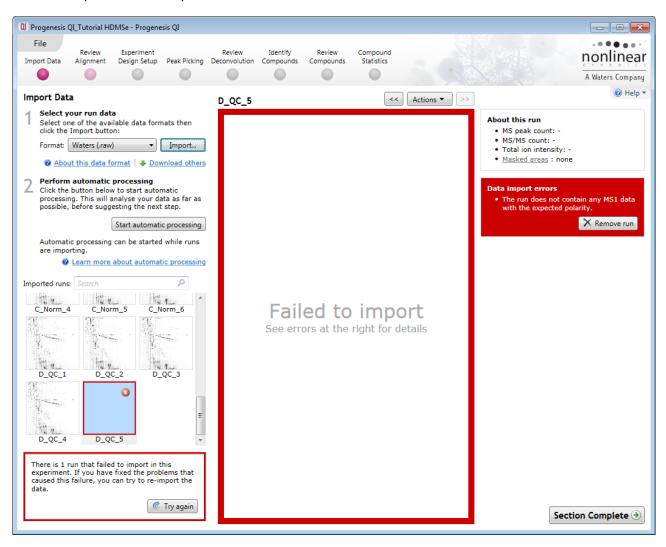
Q Processing Complete		×
Automatic process Time taken: 28 seconds	ing failed.	
A Importing runs:	19 of 19 processed 1 failed to import	
X Selecting reference:	All reference candidates failed to import	
Aligning runs:	Unable to start.	
Creating design:	Unable to start.	
Peak picking:	Unable to start.	
		Close

In this case, the processing dialog has halted indicating that all of the alignment reference candidates failed to import. To proceed restart the process with other runs.

Finally if you have chosen to select the Alignment Reference from a number of QC runs and one of them fails to import then processing will continue as there are alternative runs that did load that can be automatically selected as the reference.

	ing complete (with warnings).	
Time taken: 10 minutes 4	43 seconds	
A Importing runs:	23 of 23 processed	
	🔺 1 failed to import	
Selecting reference:	D_QC_4	
	🔺 1 candidate failed to import	
 Aligning runs: 	21 of 21 processed	
 Creating design: 	Created	
Peak picking:	6137 compound ions found	

An example of a failed import is shown below



In this example the run has the wrong polarity compared with the other runs. You can right click on the run and remove it or just click on Remove run to the right of the main display.

Click Start automatic processing to start the automatic alignment of your runs.

Note: At this stage you have the option to Review the Chromatography or go straight to the review of the Automatic Alignment of your data.

Appendix 3: Licensing runs (Stage 3)

When setting up a **New experiment** if you are evaluating Progenesis QI with unlicensed runs then the licensing page will open after **Import Data section**.



If you already have a programmed dongle attached to your machine then the License Runs page will not appear.

To use this page to License your Runs you must first either obtain an 'Evaluation' Licence Code from a Sales Person or purchase a licence code directly.

Each code will allow you to license a set number of runs.

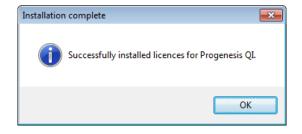
The runs in your experiment will be listed as shown below.

To activate license(s) for the selected runs enter the code in the space provided and click **Use Licence code**.

Note: you will need an internet connection to use this method.

File Review Experiment nport Data Licensing Alignment Design Setup	Review Identify Review Compound Peak Picking Deconvolution Compounds Compounds Statistics		nonline
			A Waters Comp
ongle License Runs			
This installation is currently restricted to analyse icensed runs only.	Run name	Licence state	License / this run
To license your runs, you need an evaluation or	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	
ease licence code which can be obtained from	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	
a sales representative.	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	
Once licensed, vour runs can be analysed on	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	v
any installation of the software. The licence is	$\label{eq:c:Users} C: \label{eq:c:Users} C$	Unlicensed	
automatically included when archiving an experiment.	$\label{eq:c:Users} C: \label{eq:c:Users} C$	Unlicensed	v
1	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	
f your runs have been licensed on another computer, click here to make the licences	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	
available on this computer.	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	
f you have one, you can open a licence file to	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	v
nstall.	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	v
f you have just installed a dongle, click here.	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	v
, <u>-</u>	C:\Users\andy.bothwick\Documents\Customer Data\Progenesis QI v2.2_Demo Suites_Tutorial\Progenesis	Unlicensed	v
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A message confirming successful installation of your licences will appear.



Click **OK**, the view will update and Alignment, the next stage in the workflow, will open with the licensed files.

Appendix 4: Manual assistance of Alignment

Approach to alignment (using tutorial data)

To place manual alignment vectors on a run (A_LD_1 in this example):

Note: this data is already well aligned so a single vector has already been placed to simulate misalignment so as to allow the demonstration of the use of manual vectors.



- 1. Click on Run A_LD_1 in the **Runs** panel, this will be highlighted in green and the reference run (D_QC_4) will be highlighted in magenta.
- 2. You will need approximately 5 alignment vectors evenly distributed from top to bottom of the whole run.
- 3. First drag out an area on the **Ion Intensity Map** (C), this will reset the other 3 windows to display the same 'zoomed' area.

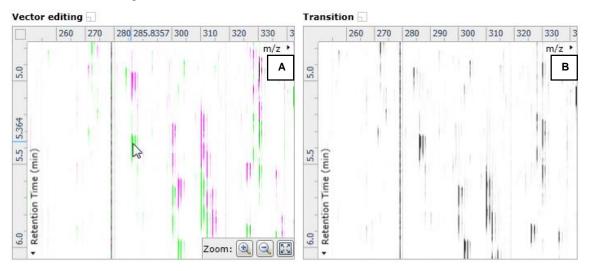
Note: when you click anywhere in the Ion Intensity Map this will **reposition the focus** and update the other views accordingly.

Note: the features moving back and forwards between the 2 runs in the **Transition** window (B) indicating the misalignment of the two runs

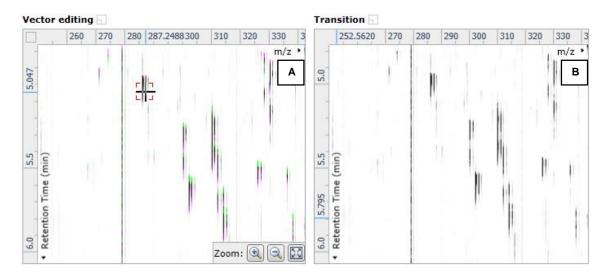
Note: the Ion Intensity Map gives you a colour metric, visually scoring of the current alignment and an overall score is placed next to the Vectors column in the table. With each additional vector this score will update to reflect the overall quality of the alignment. The colour coding on the Ion intensity Map will also update with each additional vector

Note: The **Total Ion Chromatograms** window (D) also reflects the misalignment of the 2 runs for the current Retention Time range (vertical dimension of the current Focus grid in the **Ion Intensity Map** window).

4. Click and hold on a green feature in Window A as shown below.

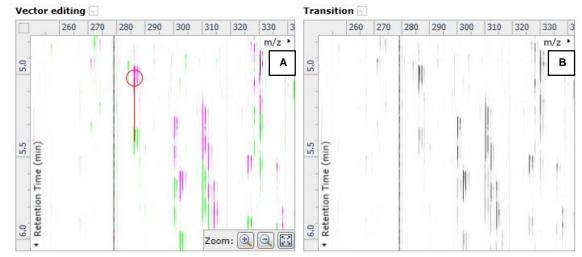


5. As you are holding down the left mouse button (depending on the severity of the misalignment), the alignment vector will automatically find the correct lock. If not, drag the green feature over the corresponding magenta feature of the reference run. The red box will appear as shown below indicating that a positional lock has been found for the overlapping features.



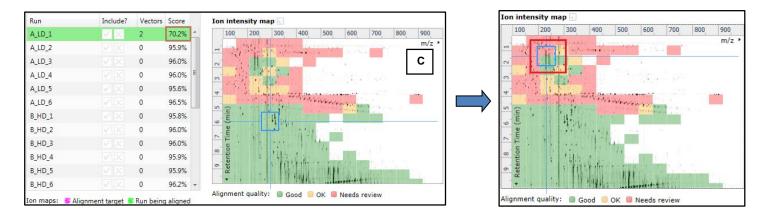
Tip: while holding down the mouse button hold down the **Alt** button. This will allow smooth movement of the cursor as the **Alt** key allows you to override the 'automatic alignment' performed as you depress the mouse button.

6. On releasing the left mouse button the view will 'bounce' back and a red vector, starting in the green feature and finishing in the magenta feature will appear.



Note: an incorrectly placed vector is removed by right clicking on it in the Vector Editing window and selecting delete vector

7. With the placement of a single manual vector the increase in the proportion of the **Ion Intensity Map** (C) showing green is reflected in the improved alignment score in the table. Now click in the Ion Intensity Map to relocate the focus in order to place the next manual vector.



8. Adding an additional vector will improve the alignment further as shown below.



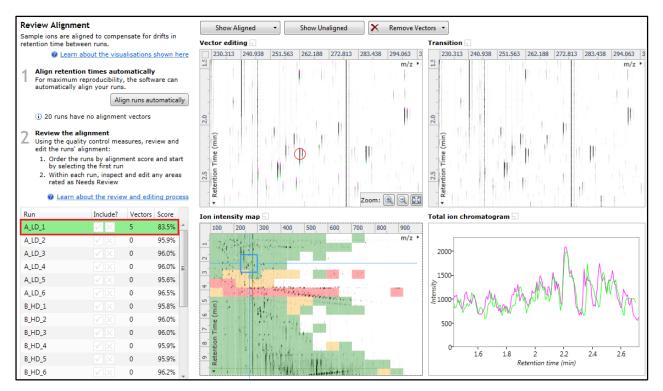
9. Repeat this process moving the focus from top to bottom on the **Ion Intensity Map** view the number you add is dependent on the length of gradient used and the severity of the misalignment. In many cases a single vector per alignment is all that is required to improve the performance of the Automatic Alignment

Note: the manual vectors are red to distinguish them from the automatic vectors which blue

Note: the number of vectors you add is recorded in the Runs table

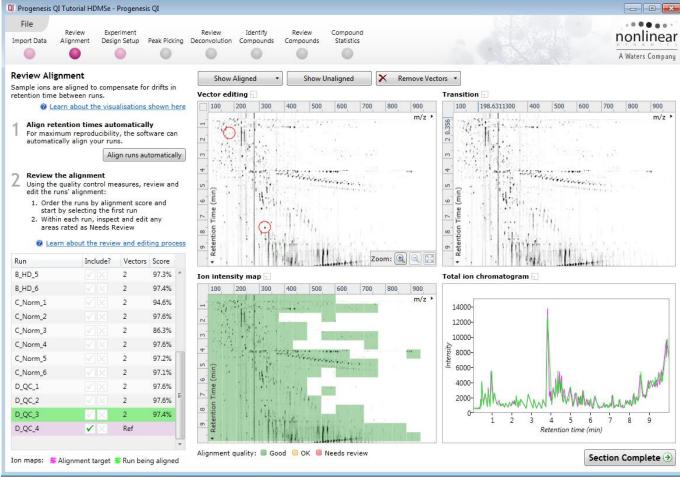
The number of manual vectors that you add at this stage is dependent on the misalignment between the current run and the Reference run. The effect of adding the manual vectors can be seen when you press show aligned

10. Repeat this process for all the runs to be aligned.



In many cases only using the Automatic vector wizard will achieve the alignment.

Also the 'ease' of addition of vectors is dependent on the actual differences between the runs being aligned.



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11. Then select Automatic Alignment to bring up the Automatic Alignment dialog and click **OK**. The automatic alignment process will begin, using the manual vectors you have added to aid in automatic vector placement.

A	Automatic Alig	nment	ĺ	x
	Choose which	runs to automatically align:		
	Run	Notes	Vectors	*
	✓ A_LD_1	run has user vectors	2	
	A_LD_2	run has user vectors	2	=
	A_LD_3	run has user vectors	2	
	🔽 A_LD_4	run has user vectors	2	
	A_LD_5	run has user vectors	2	
	🛛 A_LD_6	run has user vectors	2	
	B_HD_1	run has user vectors	2	
	B_HD_2	run has user vectors	2	
	B_HD_3	run has user vectors	2	-
			OK Cancel	

Note: the tick box next to the Run name controls whether automatic vectors will be generated for each run.

Tip: a normal alignment strategy would be: to run the automatic alignment first for all runs, then order the alignments based on score. For low scoring alignments remove all the vectors and place 1 to 5 manual vectors to increase the score then perform automatic alignment. Then review the improved alignment score.

Appendix 5: Within-subject Design

To create a **Within-subject Design** for your data set select this option on the **Experiment Design Setup** page and enter the name of the design.

In this example there are 3 Subjects (i.e. patients A, B and C) who have been individually sampled: Before Treatment: Day1 and then at 3 times following treatment: Day2, Day3 and Day4.

Q Progenesis QI Tutorial HDMSe - Progenesis QI											23
File Import Data Alignment Design Setup Peak Pi	cking De	Review Identify convolution Compound	Review ds Compounds	Compound Statistics					D	A Waters Co	I C S
Conditions Minus QC	New									0) Help 🔻
Which experiment design type do y	ou war	nt to use for this	experiment	?							
Between-subject Design Do samples from a given subject appear in only one condition? Then	A		Delete	0-0 Have you	thin-subject Des taken samples from a nder different conditi	a given		Before	During	After	
use the between-subject design.		Q Create New Experi		subject u				Before	During	Arter	
To set up this design, you simply group the runs according to the condition (factor level) of the samples. The ANOVA calculation assumes that the		Enter a name for th 3 day treatment of		sign:	<u></u>	m to	Patient X	X1	X2	X3	
conditions are independent and therefore gives a statistical test of whether the means of the conditions are all equal.	c	How do you want to Group the runs Copy an existin	manually	,		nis nas	Patient Y	Y1	Y2	Y3	
	Add con	Copy an existin	g design:	Create	design Cancel	but The	Patient Z	Z1	Z2	Z3	
				measures	vill then perform a rep ANOVA. I ANOVA is not appropi	peated					1
				because th assumptio repeated i difference reduced a condition	hardova is index the AN n of independence. Wi measures ANOVA indiv s can be eliminated or s a source of between differences (which hel nore powerful test).	NOVA ith a idual r					
				thought of paired-sar	n-subject design can be i as an extension of the nples t-test to include n between more than measures.	e					

When the design page opens use the **Add Subject** and **Add Condition** buttons to create the matrix that fits your experimental design, over typing the names as required.

Then Drag and drop the Samples on to the correct 'cell' of the matrix.

	Deconvolution Cor	dentify Review mpounds Compou patients I ×	nds Statistics	6	d		A Waters Company
Setup conditions and subjects Setup the conditions and subjects for your experiment design on the right, and then assign each of your samples to the correct subject/condition cell in the grid.		Day 1	Day 2	Day 3	Day 4	Add Condition	
 Add a column for each condition. Add a row for each subject. Drag each of your samples to the correct location in the grid. Filter samples: P 	Patient 1	A_LD_1	A_LD_2	A_LD_3	A_LD_4		
C_Norm_2	Patient 2	B_HD_1	B_HD_2	B_HD_3	B_HD_4		
C_Norm_3	Patient 3	C_Norm_1	sere Cample	Select Sample	<u>Select Sample</u>		
C_Norm_5	Add Subject			-			
							Section Complete $ i$

You can create additional Experimental Designs using the New tab

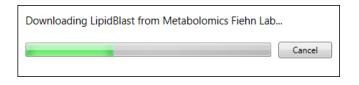
All of these Experimental Designs are available at all the following stages in the Progenesis QI workflow.

Appendix 6: Using LipidBlast for Compound Identification

When using LipidBlast for the **first** time you will require internet access to download the LipidBlast databases. Click on the download link.

QI Progenesis QI Tutorial HDMSe - Progenesis QI File Import Data Alignment Design Setup Peak Picking	Review Identify Review Compound Deconvolution Compounds Compounds	nonlinear A Waters Company
Identify Compounds Select your identification method: ↓ LipidBlast ♦ About this method ↓ Download others ▲ Before performing any LipidBlast searches, you'll need to download the databases. ♦ Download the LipidBlast databases		Ø Help ▼
 Filter the compounds Using the list below, filter the compounds to show only those you want to identify. Set the search parameters Enter the mass error tolerances for matching each compound and its fragments: 	Image: State of the state	
Precursor tolerance: 5 ppm Fragment tolerance: 5 ppm Report isomers as a single search hit Search for identifications After searching, identifications will be assigned	Lab, University of California, Davis. 10 460 480 500 For more information about LipidBlast, please visit the <u>Fiehn Lab's</u> LipidBlast project. Cancel	520 540 560
to the relevant compounds automatically.		

Dialog will open acknowledging the source of the LipidBlast libraries, click Download.



When performing a LipidBlast search the following parameters can be set:

Precursor tolerance

Set the allowable threshold (in ppm or Da) for the difference between the database neutral mass and your measured neutral mass (default is 5ppm).

Fragment tolerance

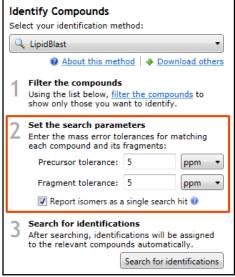
Set threshold (in ppm or Da) that determines whether a database ms/ms peak is considered to match a measured peak. The difference in m/z must fall below the threshold to be considered a match.

Report isomers as a single search hit

The search will merge hits that differ only in double bond positioning and/or geometry thus reporting isomers as a single search hit.

For example, if a search returns two identifications, GPCho(16:0/20:3(8Z,11Z,14Z)) and GPCho(16:0/20:3(5Z,8Z,11Z)), these will be reported as a single hit GPCho(16:0/20:3). When not selected, all lipid forms will be returned as separate search hits.

Having set the parameters click Search for identifications



On performing the search you will get a 'mirror plot' where the upper half represents the measured/observed fragmentation and the lower half shows the currently matched fragmentation spectrum in the database.

File port Data	Review Alignment	Experiment Design Setur	Peak Picking	Review Deconvolu	v Identify ution Compounds		Compound Statistics				nonlinea
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🕹 LipidBlas	t		•	1							
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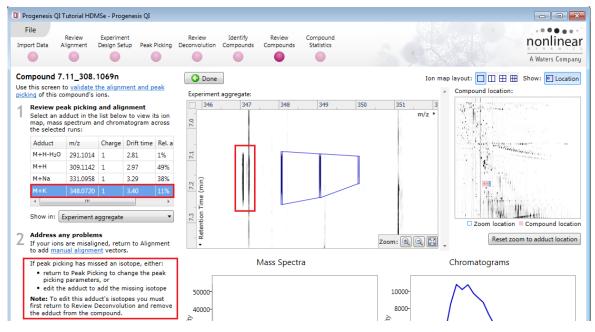
Note: a matched fragment is indicated in red, spanning the x axis. If you hold the cursor over a matched fragment details of the measured and database m/z are reported as well as the mass difference.

Where fragmentation data is available then the order will be based on the fragmentation score. In the absence of fragmentation data the score will be based on mass error and Isotope similarity.

Note: LipidBlast does not contain structural data. Clicking on the URL in the in the **Link** field, of the possible identifications table, takes you to Lipid Maps where a structural diagram is available.

Appendix 7: Editing an adduct to add a missing isotope

When using the Compound Validation view you find an example where the Peak picking has missed an isotope for an adduct ion as shown below. As shown below the monoisotopic peak for the M+K has not been detected.



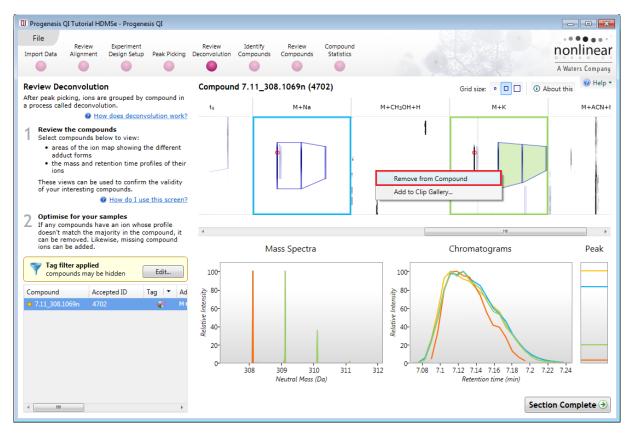
For the purposes of explaining the processes involved in editing this adduct we have (on purpose) deconstructed the M+K adduct detection.

As the note on the screen suggests you must first return to the **Review Deconvolution** screen and remove the adduct from the compound. Before doing this, click **Done** and create a tag for the compound you are about to change. Then filter so that only this compound is displayed at the **Review Compounds** stage.

I Progenesis QI Tutorial HDMSe - Progenesis QI			
File Review Experiment Import Data Alignment Design Setup Peak Picking	Review Compounds Compounds Statistics	A GAN	A Waters Company
Review Compounds	Find a compound: Search P	Filter compounds 👻 Filter is active	Ø Help ▼
Using this screen, you can find the compounds of interest in your experiment.	Compound Neutral mass m/z z R	letention time Drift time Peak Width Tag 💌	Accepted ID Identifications Anov
Create a shortlist to review In the table, sort and filter the compounds based	• 7.11_308.1069n 308.1069 347.0700 1 7	7.11 3.40 0.18 🗳	4702 1 < 1.1
on their measurements, to generate a shortlist for further review.	Q Filter the compounds		
How are the measurements calculated?	Create a filter		
To sort the table by a given value, simply click the relevant column header.	Show or hide compounds based on a selection of the to create the filter. For more guidance, please see the		
2 Review the compounds For each compound of interest, inspect the ions'	Available tags: S	how compounds that have all of these tags:	
alignment and peak picking: Review selected compound	Not identified (5082 compounds) Anova p-value ≤ 0.05 (1258 compounds)	My Compound (1 compound)	
You can also double-click to review a compound.	S_Plot_B (87 compounds)	how compounds that have at least one of nese taos:	B_HD
3 Choose the correct identifications For each compound, select one of its possible identifications as the accepted one.	Separated by drift time (155 compounds) S_Plot_A (187 compounds)	icae toga.	
To speed this up, you can <i>automatically</i> accept identifications in compounds where only one of the possible identifications has:	Confirmed Compounds (9 compounds)	lide compounds that have any of these tags:	
Score ≥ 48.0 Accept identifications			
4 Export data for further processing By exporting your data to external tools, there's no limit to your analysis.	Clear the filter	OK Cancel	
Export to pathways tool	4.333333 - -		
	2.166667 —		
Experiment design	4		Þ
Review your data from a different perspective: Current design: Minus QC			Section Complete $\textcircled{3}$

Now click Review Deconvolution on the workflow

In this view the 3 Adducts are displayed. Right click on the M+K adduct and select **Remove from Compound**.



The table will update to show 2 compounds (both tagged). The second one is based on the single adduct M+K.

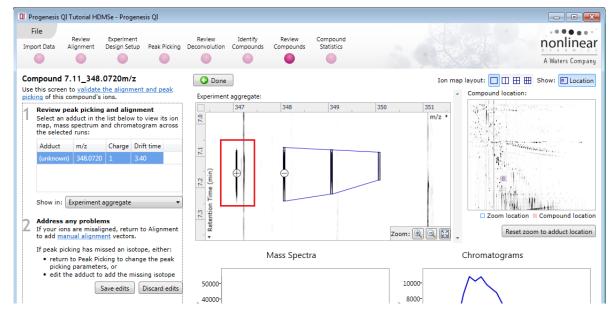


Now click on **Review Compounds.**

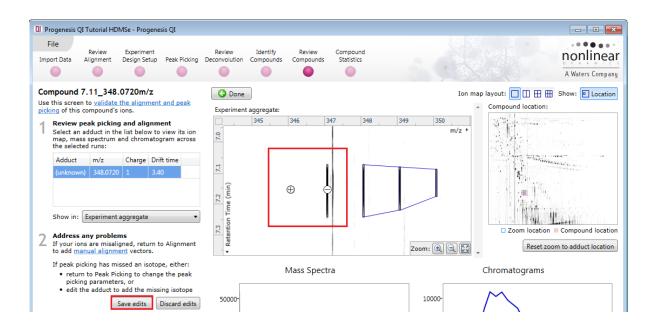
Select the compound with the 'unknown' Neutral Mass and click Review selected compound

•							•		
QI Progenesis QI Tutorial HDMSe - Progenesis QI									×
File Experiment Import Data Alignment Design Setup Peak Picking I	Review Identify Deconvolution Compounds	Review Comp Compounds Stati						A Waters Con	eal
Review Compounds	Find a compound: Search	له h	2	Filter compo	ounds 👻 Filter is a	active		٢	Help
Using this screen, you can find the compounds of interest in your experiment.	Compound N	leutral mass m/z	z Rete	ention time Dri	ift time Peak Width	Tag 🔹	Accepted ID	Identifications	Anov
Create a shortlist to review	• 7.11_348.0720m/z <	unknown> 348.0720				\$			< 1.1
In the table, sort and <u>filter the compounds</u> based on their measurements, to generate a shortlist for further review.	• 7.11_308.1069n 30	08.1069 309.1142	1 7.11	. 2.9	0.18	@	4702	1	< 1.1
How are the measurements calculated?									
To sort the table by a given value, simply click the relevant column header.	•	III							•
	Compound 7.11_348.07	20m/z:							
2 Review the compounds For each compound of interest, inspect the ions'	Compound abundance	Possible identification	s 3D N	1ontage Drift	time montage				
alignment and peak picking: Review selected compound		C_Norm			A_LD		B_HI	D	
You can also double-click to review a compound.	-								

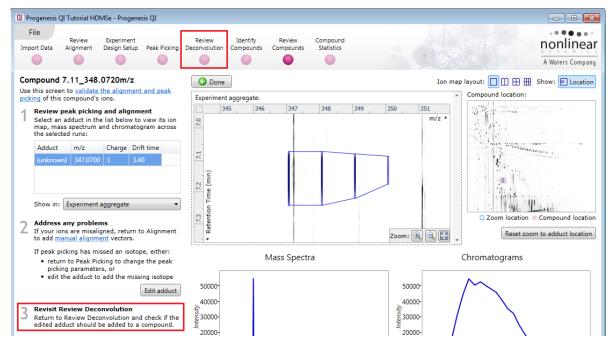
Now click on **Edit Adduct**. A **plus and minus** will appear on the experiment aggregate view. Click on the **plus** to add the missing isotope.



The view updates to show the minus sign now on the added isotope. Click on Save edits to accept the edit



The view will update to show the added isotope and a note will appear indicating that you should return to Review Deconvolution to check if the edited adduct should be added to a compound . Click on **Review Deconvolution** on the workflow.



Right click on the edited adduct and select Add to Compound

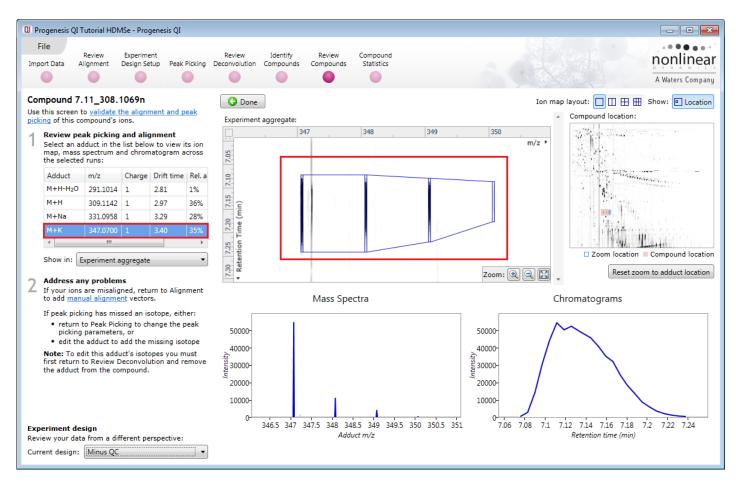
Review Deconvolution	Compound 7.11	308.1069n (4	702)			Grid size:	🗆 🗌 🛈 Abo	ut this
After peak picking, ions are grouped by compound in a process called deconvolution.	M+H-2H ₂ O	M+H-H ₂ O	M+H	M+NH4	M+Na	M+CH3OH+H	M+K	M+ACN+H
Review the compounds Select compounds below to view: areas of the ion map showing the different adduct forms the mass and retention time profiles of their ions		8				to Compound to Clip Gallery		

The compound and its details will update to and the compound table will now show a single compound.

QI Progenesis QI Tutorial HDMSe - Progenesis QI		[- • ×
File Experiment Import Data Alignment Design Setup Peak Picking	Review Deconvolution	<u>• •</u>	Maters Company
Review Deconvolution	Compound 7.11_308.1069n (4702)	Grid size: 😐 🗆 🛈 About t	this 🕜 Help 🔻
After peak picking, ions are grouped by compound in a process called deconvolution.	M+H-2H ₂ O M+H-H ₂ O M+H	M+NH4 M+Na M+CH3OH+H M+K	M+ACN+H
 Review the compounds Select compounds below to view: areas of the ion map showing the different adduct forms the mass and retention time profiles of their ions These views can be used to confirm the validity of your interesting compounds. Whow do I use this screen? 			
Optimise for your samples If any compounds have an ion whose profile doesn't match the majority in the compound, it can be removed. Likewise, missing compound ions can be added.	Mass Spectra	"' Chromatograms	Peak
Tag filter applied compounds may be hidden Editum Compound Accepted ID Tag Image: Adit of the second	100- 80- 60- 40-	100- 00- 00- 00- 00- 00- 00- 00-	

Now click on **Review Compounds** and click **Review selected compound** with the edited compound selected.

When you now click on the M+K adduct it contains 4 isotopes and its m/z has altered accordingly to reflect the addition of the isotope.



Appendix 8: Exporting compound identities to IPA

IPA (Ingenuity Pathway Analysis): is a commercial web-based software application for the analysis, integration and interpretation of data derived from any experiments that generate gene and chemical lists with expression data, such as metabolomics and proteomics.

Before exporting data to IPA, make sure that you can launch IPA using the default browser on your system. The plug-in relies on the browser to upload the Progenesis-exported data to IPA.

Note: the use of IPA requires a licensed copy, for licensing details please visit the <u>IPA website</u>.

First select the compound data to export to the pathways tool using tag filtering to 'focus' the set to export.

Click Export to pathways tool and select IPA and click Next.

Q Progenesis QI Tutorial HDMSe - Progenesis QI					- • ×
File Review Experiment Design Setup Peak Picking Deconvolution Compounds Compounds					A Waters Company
Review Compounds Find a compound: Search	P Filter	r compounds 👻 F	ilter is active		🔞 Help 🔻
Using this screen, you can find the compounds of interest in your experiment. Compound Neutral mass	m/z z Reten	Filter compounds	ig 💌	Accepted ID	Identifications
Create a shortlist to review Q Export Pathways Information		×	•	7847292	1
In the table, sort and filter the con on their measurements, to general Select a pathways tool			-	49854487	4
for further review. Choose a pathways tool from the list below. You ca	n find out more or downloa	ad new 0.18	e	4702	1
		0.14	. 🔶	9357	1
To sort the table by a given value, the relevant column header.		0.14	•	14708992	1
IPA: Ingenuity Pathway Analysis		▼ 0.27	· 🔶	7847488	1 *
2 Review the compounds For each compound of interest, ins alignment and peak picking: Review select	this plugin 🖶 Download .	other plugins			Þ
You can also double-click to review		indig		B HO	
3 Choose the correct identificatio For each compound, select one of i identifications as the accepted one To speed this up, you can automat identifications in compounds where the possible identifications has: Score ≥ 50.0 Accept				b_rit	,
4 Export data for further process By exporting your data to external no limit to your analysis.	< Back Next >	Cancel			
Export to pathways tool	*				
Experiment design Review your data from a different perspective:					Þ
Current design: Minus QC				Sectio	on Complete 🏵

Export of the Accepted IDs only is

recommended then select the appropriate identifier type used for compounds in the experiment.

Note: for compounds with different identifier types, filter the compound list to contain only compounds with single identifier type at a time, then perform the export operation and merge the resulting lists in IPA.

Export Pathways Information	
Configure your export Choose which identifications to export and the type of analysis you want to perform.	
Select which identifications to export:	
Accepted IDs only (recommended)	
Select the identifier type used for compounds in the experiment:	
Human Metabolome Database (HMDB)	-
CAS Registry Number KEGG	
Human Metabolome Database (HMDB)	
PubChem CID	

Finally select the way you want to export the compounds to IPA.

If you are performing over-representation analysis or other types of analysis that do not require the compound expression data then you can make use of the **Create a list** option to export data to IPA.

If you require to perform enrichment analysis or other types of analyses that use compound expression data, select the option to **Upload expression dataset** to IPA.

QI Export Pathways Information			
Configure your export Choose which identifications to export and the type of analysis you want to perform.			
Select which identifications to export:			
Accepted IDs only (recommended)			
Select the identifier type used for compounds in the experiment:			
Human Metabolome Database (HMDB)			
Select the way you want to export the compounds to IPA:			
Upload expression dataset			
For expression data, choose two experimental conditions that you would like to compare.			
Baseline: Comparison:			
C_Norm			
If greater, gives a negative fold change.			
ALD			
□ B_HD			
< Back Export compounds to IPA Close			

Note: You need to select the type of compound identifier used by at least one compound whose expression data you want to export. If there are compounds with different identifier types, you will be able to select additional identifier types in IPA after the dataset is uploaded.

The operation exports data from a single observation at a time - select the two experimental conditions (taken from the current **experiment design as set in Progenesis QI** you wish to compare as that observation).

Click Export compounds to IPA .

v) 0 L	apps.ingenuity.c	∩
INGEN	IUITY ⁻	
Welcome! Pl	ease login	Contact Customer Support
Email Password	smith@work.com	Customer Support Phone: 650.381.5111 Hours: 6am - Spm (PST)
	Remember my password LOG IN Sign Up Forgot Password	Monday - Friday (excluding holidays) support@ingenuity.com For Product and Sales related inquiries contact: 650.381.5056 sales@ingenuity.com

If you have access to a licenced copy of IPA then log in

IPA will open displaying the imported data from Progenesis.

<u>E</u> dit <u>V</u> iew <u>W</u> indow	Help	Provide Feedback Support Janucz Nykiel Close
	Genes and Chemicals Diseases and Functions Pathways a	and Tox Lists
vž		
V 🏹	Enter gene names/symbols/IDs or chemical/drug names here	SEARCH Advanced Search 👰
set Upload - New Datase	t 2015-05-11 07:35 AM	Dataset Upload Workflow Instructions
elect File Format:	Ingenuity File Format A or B	Defe Unite ed Workflow
Contains Column Header:	○ Yes	Data Upload Workflow
elect Identifier Type:	Human Metabolome Database (HMDB) Specify the identifier type f	Use Dataset Upload to import your dataset file into IPA.
		Biomarker Filter, Molecular Tox and Core Analyses, Review
Array platform used for exp	periments: Not specified/applicable Select relevant array platfo	the different type of analyses and see which one best fits
Jse the dropdown menus	to specify the columns that contain identifiers and observations. For observations, selec	ct the appropriate expression value type. your needs.
		1. To upload a dataset file, click here.
aw Data (567) 🛛 Dataset Su	mmary (182) \	
,		
ID 👻	Observation 1 💌	Look jn: 🗀 Multiple Rank. 🔻 வ 🟠 😂 😂
	Fold Change 🔻	Batch (mult-timepoint)
		Afry_with_p-value_and_fold.txt UL_with_Fold and_Normalized.txt
1 HMDB01988	Infinity	
2 HMDB05015	1.72884040538655	
3 HMDB40639 4 HMDB40285	3.98236765102672 2.1144344578431	
5 HMDB39847	1.91509320582337	
6 HMDB05049	1.36771507748685	
7 HMDB30934	-2.37287619375142	File (jame:
8 HMDB37820	1.69294555757503	Files of Type: All Files
9 HMDB15294	-1.4569308616069	Open Cancel
10 HMDB39350 11 HMDB41326	-1.46035570178035 1.89049486451889	
12 HMDB34382	1.75866062252427	Select the dataset file from your computer and click the
13 HMDB35337	3.98452117071048	Open button.
14 HMDB36734	2.38572167550178	D. Calact Flavible format for the fla format for the
15 HMDB13809	-1.25125092715796	 Select Flexible format for the file format from the dropdown menu.
16 HMDB14821	1.08777276460426	
17 HMDB00782 18 HMDB40806	1.14588290027386 1.83433974738324	4. Select an Identifier Type from the dropdown menu. IPA
19 HMDB30926	2.51317063326778	supports many identifiers and symbols and will attempt to
20 HMDB40179	1.42264275983645	guess at the type of identifier in your dataset file. To override the selection, uncheck the option and simply select
21 HMDB13250	1.17037352363088	the most appropriate one. If more than one type of identifier
22 HMDB37712	1.50281207491669	exists in your dataset, select all appropriate ones.
23 HMDB38057	1.43917459561637	
24 HMDB40285 25 HMDB02725	1.84463305905821 1.80218245845514	GenBank
25 HMDB02725 26 HMDB29978	2.11110785701812	Select All
27 HMDB37272	1.27732135837318	Affymetrix
28 HMDB39848	1.14877655967276	Affymetrix SNP ID
29 HMDB29828	-1.35134004957123	Agilent
30 HMDB30932	1.82757219666658	CAS Registry Number
31 HMDB13300	1.38248130204499	CodeLink
32 HMDB32791	1.21282984334921	Entrez Gene (LocusLink)
		GenBank
		GenPept

You can now explore your compound expression data using the tools available in IPA. Instructions on how to manage and explore your imported data set are provided by IPA.

Appendix 9: Power Analysis

Power analysis is a statistical technique, which is used to gauge how many replicates are needed to reliably observe the abundance differences in your data. It is available through the Compound Stats section of the workflow.

To perform a power analysis of the data click on **Ask another question** at the top of the table in the Compound Stats screen. A selection of 4 tools will appear in the form of questions.

v	Principal Components Analysis Are there any outliers in my data? Does my data cluster according to my experimental conditions?
ጨ	Correlation Analysis Group my compounds according to how similar their abundance profiles are.
Ľ	Power Analysis How many replicates should I run? What is the power of my experiment?
	Adduct Abundance What adducts do my runs contain?

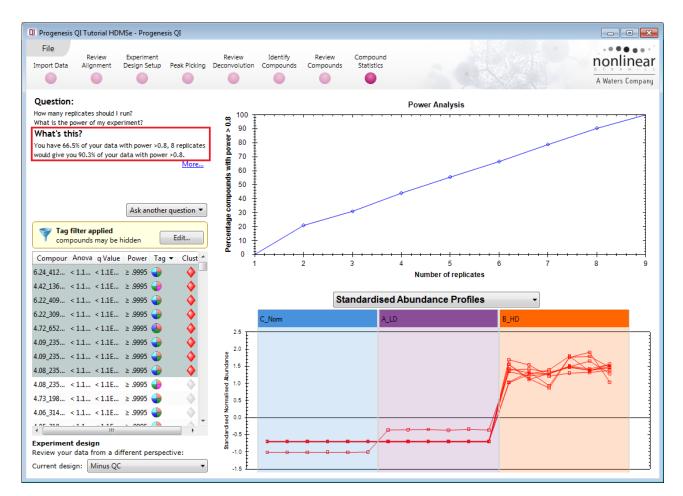
Select the option

'How many replicates should I run and what is the power of my experiment?'

It answers this question by informing you:

'How many replicates you need so that at least 80% of your compounds with a power >0.8'

Using only the compounds tagged **Anova p<0.05 (i.e. 1258 compounds),** as an example, view the power analysis.



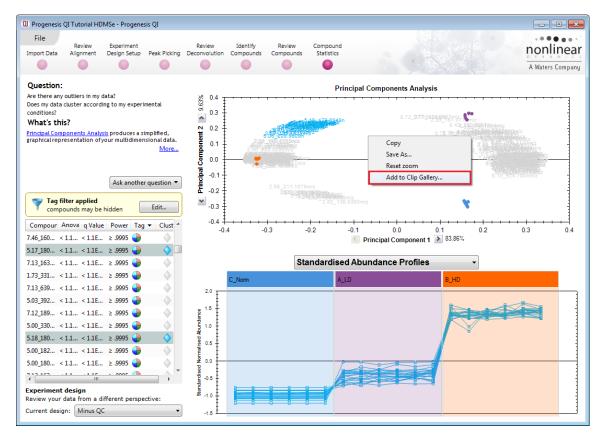
This is displayed graphically showing that 66.5% of the 1258 compounds have a power of 80% or that 8 replicates would give you 89% of your data with power > 0.8.

Appendix 10: Using Clip Gallery to Save and Export Pictures and Data

At every stage of the Progenesis QI workflow the images and data tables can be added to the Clip Gallery.

The saved images are retained as part of the experiment and are stored accordingly. This facility allows you to capture (high resolution) images that can be used in the development of specific reports and/or used as part of the process of publishing your experimental findings.

As an example of using the Clip Gallery, at the **Compound Statistics** view, displaying the PCA plot. Right click on the **Biplot** View and select Add to clip gallery....



This will open a dialog displaying what is to be saved and allows you to alter the title and provides a description of the image for later reference.

Q Add to Clip Gallery				
Enter a title and description for this clipping:				
Principal Components Analysis				
13				
43 International Control Contr				
43 Y				
-0.4 -0.4 -0.3 -0.2 -0.1 0.8 0.1 0.2 0.3 0.4 Principal Component 1 -0.045				
10.0%				
Compounds from EZinfo biplot PCA graph				
Imported Biplot details from EZinfo				
Add to Clip Gallery Cancel				
Add to Clip Gallery Cancel				

Enter details as required and click Add to clip gallery

	QI P	Progenesis QI Tutorial HDMSe - Pro	oge
		File	
To view, edit and/or export from the clip galley the gallery can be		Save	tu
accessed from the File menu.		Close	
		Export inclusion list	I.
		Export to EZinfo Open EZinfo project	æ
		Experiment properties	s i
		Show Clip Gallery	Im
Selecting an image in the gallery makes available an Actions menu that allows you to manipulate the output of the image.	×	Exit	

Item size: Item size: <th>Programme Auger</th> <th>Edit Copy Image Copy Title Copy Description</th> <th></th> <th>Data analysis performed using:</th>	Programme Auger	Edit Copy Image Copy Title Copy Description		Data analysis performed using:
Edit Copy Image Copy Title Copy Description Export Image Delete Delete Www.nonlinear.com	Programma koper	Edit Copy Image Copy Title Copy Description	nesis Ql	
Compounds from EZinfo biplot PCA graph Progenesis QI logo Progenesis QI analysis badge Imported Biplot details from EZinfo A high-resolution image that you can use in presentations and posters to A high-resolution image that you can use in presentations and posters to				www.nonlinear.com
research. research.	graph	Diplot PCA Progenesis Q A high-resoluti EZinfo use in present show the softw	ion image that you can	A high-resolution image that you can use in presentations and posters to show the software was used in your

Note: there is also the capacity to **Export all...** the images in the experiments clip gallery which creates a list of files in a folder of your choice where the file name is based on the image title.

Note: right clicking on a table and adding it to the Clip Gallery allows you to export the current content to Excel.

Appendix 11: Waters Machine Specifications and approx. timings

This appendix provides information on the time(s) taken at each stage and the total time taken to analyse a set of 22 (Phase 1) HDMSe runs on a Waters Demo Spec PC.

Machine Spec: Lenovo						
-	® CPU 2.66GHz 12core	X5650 @ 2.67GHz				
RAM: 24.0 GB						
System Type: 64-bit Op	perating System					
, , , , , , , , , , , , , , , , , , ,	0 7					
File Folder Size: Each	File Folder Size: Each file folder (.RAW): 0.75 Gig					
Analysis Stages:	Per file		Total			
Auto processed:	Import and analysis of data to peak picking		30.0min			
Import Data:	Loading of Raw data per file	1min	22min Total for 22 files			
	(re-opening at Import Data)	5s				
Alignment:	Automatic alignment of data (re-opening at Alignment)		6.0min 10s			
Peak Detection:	Automatic Detection of data		4.5min			
	(re-opening at Peak Detectio	n)	10s			
Identify Compounds:	Performing .SDF Compound	Search	5s			
	(re-opening at Identify Comp	ounds)	10s			
Total Analysis Time Ir		32.5min				
Restoring Progenesis QI tutorial HDMSe experiment from archive			20s			