

Agilent MassHunter Workstation Software

Quantitative Analysis Quant Classic Familiarization Guide

Notices

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1 Introduction

Introduction

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1 Introduction

Choosing Quantitative Analysis Desktop Icons

Choosing Quantitative Analysis Desktop Icons

Quantitative Analysis offers desktop icons for the **Classic** user interface and the **Quant-My-Way** user interface. The Classic user interface has a look and feel similar to the user interface offered in Quantitative Analysis, with tools and options located in a menu bar. The **Quant-My-Way** user interface has a modern ribbon, with tools and options located on tabs and ribbons instead of in a menu bar. You can select to install the **Classic** user interface desktop icons, the **Quant-My-Way** user interface desktop icons, or a mix of both.

Depending on how the Quantitative Analysis program was installed, you may find several different icons on the desktop, each representing a different instrument type. When you start the Quantitative Analysis program from these icons, the default values and some of the features are customized to the selected instrument type.

When you click any of these icons, the full name of the installed program is displayed. Make sure you choose the icon that matches the type of data you want to analyze.

This Familiarization Guide follows the Classic user interface.

Before You Begin These Exercises

Be sure the data files you will be using as you complete the exercises in this document are on your PC.

- If the default MassHunter Quantitative Analysis Software Supplemental installation was completed, the data files needed for these exercises should be present in **MassHunter/Data/QuantExamples**.
- If the default MassHunter Quantitative Analysis Software Supplemental installation was not completed, you can copy the data from the installation media (Supplemental/MassHunter/Data/QuantExamples) to any location on your PC.

Task 1. Set Up a New Batch 8

Task 2. Set Up a New Method for the Batch 11

Task 3. Set Up Target Compounds 14

Task 4. Set Up Quantitation 16

Task 5. Set the Integrator 21

Task 6. Analyze and Save the Batch 23

In this exercise, you set up a quantitation method for a batch of acquired data files. You carry out the exercise with the **DrugsOfAbuse** data files (See **"Before You Begin These Exercises"** on page 6) and learn how to perform the following tasks:

- Set up a Batch Table containing unknown sample and calibration data files for drugs of abuse: amphetamine, cocaine, methamphetamine, and MDMA.
- Set up a new quantitation method based on the calibration standard of the highest concentration.
- Set up target compounds.
 - View the MRM transitions and chromatographic parameters for the compounds in the data file.
 - Set up an internal standard for each of the compounds.
- Set up quantitation for the method.
 - Create levels from calibration samples.
 - Set up qualifier ions and the calibration curve.
- Quantitate the batch and save the results.

Task 1. Set Up a New Batch

Task 1. Set Up a New Batch

In this task, you set up a Batch Table containing data files for three unknown samples and several calibration samples of drugs of abuse: amphetamine, cocaine, methamphetamine, and MDMA.

1 Click the Quantitative Analysis (QQQ) icon on your desktop. Quantitative Analysis program.

When you first use the program, the default layout appears, as shown in Figure 1.

📅 Agilent MassHunter Quantitative Analysis	
File Edit View Analyze Method Update Report Tools Help	
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Batch Table	
Sample: 🝸 🔍 📕 Sample Type: 👻 Compound: 💓	- 🖬 ISTD:
Sample Transmitter Sample Transmitter Type Level Acc. Date-Time	
Compound Information	Calibration Curve
	→ + + ISTD QC CC
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x101	\$ x10 ²
0.5	
0.8-	2 0.6-
0.7-	0.4-
0.6-	0.2-
0.5-	0-
0.4-	-0.2-
0.3-	-0.4-
02-	-06-
01	
0.14	-0.8-
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9	-1-4 -80 -60 -40 -20 0 20 40 60 80 Concentration
	TWficm

Figure 1. Default layout

You can also access the program by clicking Programs > Agilent > MassHunter Workstation > Quantitative Analysis (QQQ) from the Start menu.

Different features are available when you are working with QQQ data.

2 Exercise 1: Set Up and Quantitate a Batch of Acquired MRM Data Files Task 1. Set Up a New Batch

If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new batch.

Restore <u>D</u>efault Layout

- 2 Click File > New Batch. The system opens the New Batch dialog box.
- 3 Navigate to the folder \Your Directory\DrugsOfAbuse\.
- 4 Type the batch file name *iii*_Test_01 and click Create.
- 5 All Samples should be selected. Click OK to add them to the batch.

The **Batch Table** is no longer empty. It now contains the calibration, QC, and unknown samples. See **Figure 2**.

Add Samples	? <mark>x</mark>
Batch Folder: D:\Mass	Hunter\Data\Qua
File name	Sample name 🔺
CMAMBlk_01.d	Blank-1
CMAMCal_L1.d	Calib-L1
CMAMCal_L2.d	Calib-L2
CMAMCal_L3.d	Calib-L3
CMAMCal_L4.d	Calib-L4
CMAMCal_L5.d	Calib-L5
CMAMQC_L2.d	QC-L2
CMAMQC_L4.d	QC-L4
CMAMSam_01.d	Sample-1
<	C
Browse to Copy Sample	×s
Translate MSWS Sampl	es
Select All OK	Cancel

Task 1. Set Up a New Batch

Note that only three of the files are unknown samples, one is a blank, five are calibration files at different calibration levels, and two are QC samples.



Figure 2. Batch Table containing Drugs of Abuse samples before quantitation

2 Exercise 1: Set Up and Quantitate a Batch of Acquired MRM Data Files Task 2. Set Up a New Method for the Batch

Task 2. Set Up a New Method for the Batch

This task shows you how to set up a new quantitation method based on the calibration data file with the highest concentration of sample.

1 Use the cursor to highlight the calibration standard that has the highest concentration level, as shown in the figure below.

Using a sample with strong signals for the compounds, such as a high-concentration calibration sample, lets the program create a method with the appropriate retention times and qualifier ratios.

📅 Agil	ent A	AassHur	iter Qu	antitat	ive Analysis - Drug
<u>E</u> ile	<u>E</u> dit	<u>V</u> iew	<u>A</u> nalyze	e <u>M</u> eth	od <u>U</u> pdate <u>R</u> ep
: 🛅 🖻	, 📕	l Gal Ç	,≣ <u>A</u> nal	/ze Bat	ch 🛛 🥑 🕴 Layout:
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: Sam	ple:		Sampl	е Туре:	<all> ▼ C</all>
			Sam	ple	
۲	7	Name	Туре	Level	Acq. Date-Time
		Blank	Blan		5/12/2006 1:48
		Calib-	Cal	L1	5/12/2006 1:51
		Calib-	Cal	L2	5/12/2006 1:54
		Calib-	Cal	L3	5/12/2006 1:57
		Calib	Cal	L4	5/12/2006 2:00
Þ		Calib-	Cal	L5	5/12/2006 2:03
		QCL	QC	L2	5/12/2006 2:06
		QC-L	QC	L4	5/12/2006 2:09
		Samp	Sam		5/12/2006 2:12
		Samp	Sam		5/12/2006 2:15
		Samp	Sam		5/12/2006 2:18

Task 2. Set Up a New Method for the Batch

2 Click **Method > Edit** to switch to method editing mode.

The **Method Tasks** appear in the column to the left of the View, as shown in **Figure 3**.

Note that Figure 3 shows the default layout for method editing.

If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new method in the next step.

📅 Agilent MassHunter Quantitative Analysis	- Method	- <c:\us< th=""><th>ers\cm.</th><th>TWI\De:</th><th>sktop\D</th><th>rugsOfA</th><th>buse_</th><th>B0500_</th><th>update</th><th>d\Drug</th><th>JsOfAbu</th><th>ise_B05</th><th>00\Quai</th><th>ntResult</th><th>s\iiii_Test</th><th>_01.bat</th><th>ch.bin></th><th></th><th></th><th></th><th>. • 💌</th></c:\us<>	ers\cm.	TWI\De:	sktop\D	rugsOfA	buse_	B0500_	update	d\Drug	JsOfAbu	ise_B05	00\Quai	ntResult	s\iiii_Test	_01.bat	ch.bin>				. • 💌
File Edit View Analyze Method Upda	ite Report	t Tools	Help																		
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Method Tasks 🗸 🗸	Method Ta	able																			
New / Open Method	Time	Segment	t 🖛 🛛	<all></all>		▼ ⇒	G	ompour	nd: 🔄]				Reset T	able View						
Method Setup Tasks	Samp	le																			
K MRM Compound Setup		Name		Di	ata File		1	Туре		l	.evel	A	cq. Meth	od File	Acq. Da	te-Time	•				
K Retention Time Setup		alib-L5		CMAM	Cal_L5.0	I Ca				L5		AF	Clautot	ine.m	5/12/2000	5 2:03					
或 ISTD Setup																					
🤣 Concentration Setup																					
🕂 Qualifier Setup																					
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Exit	4-															1	1 1	1			-0.6
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Outlier Setup Tasks	0-															$ \downarrow $	U				0
Advanced Tasks		0.1 0	0.2 0.3	0.4 0	5 0.6	0.7 0.	8 0.9	1	1.1 1.	2 1.3	1.4 1	.5 1.6	1.7 1	.8 1.9	2 2.1	2.2	2.3 2.4	2.5	2.6 2.7 Acquisitio	2.8 on Time	2.9 (min)
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		0.0		v.		_ 0.		0.0	0.00	0.4	0.40	0.0	0.00	0.0	0.00	0.7	0.70	0.0	0.00	0.0	0.00
																0 Co	mpound	s (0 tota	I) 0 ISTE) (0 total	I) TWI\cm

Figure 3. Method Edit mode

Task 2. Set Up a New Method for the Batch

3 Under Method Tasks in the sidebar to the left of the Method Table, click New/Open Method > New Method from Acquired MRM Data.

You can also click **Method > New > New Method from Acquired MRM Data**.

Agilent MassHunter Quantitative Analysis (for QC	QQ) - Method - <c:\masshunter\data\quantexamples\< th=""></c:\masshunter\data\quantexamples\<>
File Edit View Analyze Method Update Rep	port Tools Help
🗄 🛅 🗁 🛃 📭 💭 Analyze Batch 🔻 🕢 🤅	Layout: 🔜 🔛 🔠 🖾 📝 Restore Default La
Method Tasks 🗸 🗸	Method Table
New / Open Method	🕴 Time Segment: 🖛 < All> 🔍 🔿 🔿
📑 New Method from Acquired MRM Data	Sample
New Method from Acquired Scar New Method from A	Acquired MRM Data
New Method from Acquired Scan Data with Libr	CMAMCal_L5.d Cal

- 4 Select **No** to the prompt **Would you like to apply this method to the batch?** The system displays a **New method From Acquired Data** dialog box.
- 5 Click **CMAMCal_L5.d** and click **Open** to import acquisition method information.

New Method	from Acquired Data		×
Look in:	\mu DrugsOfAbuse	- G 👂 📂 🛄-	
æ	Name	Date modified	Туре 🔺
	CMAMBIk_01.d	12/10/2013 2:57 PM	File fol
Recent Places	\mu CMAMCal_L1.d	12/10/2013 2:56 PM	File fol
	CMAMCal_L2.d	12/10/2013 2:56 PM	File fol
	🕌 CMAMCal_L3.d	12/10/2013 2:56 PM	File fol 🗏
Desktop	\mu CMAMCal_L4.d	12/10/2013 2:56 PM	File fol
<u> </u>	\mu CMAMCal_L5.d	12/10/2013 2:56 PM	File fol
	CMAMQC_L2.d	12/10/2013 2:56 PM	File fol
Libraries	CMAMQC_L4.d	12/10/2013 2:56 PM	File fol
	👪 CMAMSam_01.d	12/10/2013 2:56 PM	File fol
	🍌 CMAMSam_02.d	12/10/2013 2:56 PM	File fol
Computer	👪 CMAMSam_03.d	12/10/2013 2:56 PM	File fol
	DOA_ReportMethod.m	12/10/2013 2:48 PM	File fol 🔻
	•		+
Network	Object name:	-	Open
	Objects of type:	-	Cancel
			Help

Task 3. Set Up Target Compounds

Task 3. Set Up Target Compounds

With this task, you learn to inspect the MRM transitions and the RT data for the new quantitation method, which you can change for individual target compounds. You also learn to set up an ISTD compound for each target compound.

1 Under Method Tasks in the sidebar to the left of the Method Table window, click Method Setup Tasks > MRM Compound Setup.

The compound names associated with MRM transitions are entered in the acquisition method. By default, the largest signal is chosen as the quantifier ion.

Agilent MassHunter Quantitative Analysis - [New Meth	od]													
File Edit View Analyze Method Update Report T	ools	Help												
🗅 🗁 📕 📭 💭 Analyze Batch 🕜 🗄 Layout 🔜 🔢 🛄 🛆 🐼 Restore Default Layout														
Method Tasks 👻 🗙	Me	thod Tal	ble											
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New Method from Acquired MRM Data		Sample	e											
New Method from Acquired Scan Data			Name	Data File	Тур		Level	Acq. Method F	ile Acq. Date-Time					
New Method from Acquired Scan Data with Library Se	-	> CI	MAMCal_L5.d	CMAMCal_L5.	d									
New Method using Manual Setup														
Open Method from Existing File			Name	TS	Transition		Scan	Туре	Precursor Ion	Product Ion	RT	Ion Polarity		
			Amp	1	136.2 -> 91.4	MRM		Target	136.2	91.4	2.102	Positive		
Open Method from Existing Batch		5	Amp-d5	1	141.1 -> 93.4	MRM		ISTD	141.1	93.4	2.078	Positive		
Method Setup Tasks			Cocaine	1	304.1 -> 182.0	MRM		Target	304.1	182.0	2.449	Positive		
			Cocaine-d3	1	307.1 -> 185.0	MRM		ISTD	307.1	185.0	2.450	Positive		
K MRM Compound Setup			MDMA	1	194.2 -> 163.3	MRM		Target	194.2	163.3	2.269	Positive		
/K Retention Time Setup			MDMA-d5	1	199.2 -> 164.3	MRM		ISTD	199.2	164.3	2.269	Positive		
A 1979 A 1			Meth	1	150.1 -> 119.3	MRM		Target	150.1	119.3	2.239	Positive		
IST D Setup		1	Meth-d5	1	155.2 -> 92.3	MRM		ISTD	155.2	92.3	2.233	Positive		
🧷 Concentration Setup														
🛣 Qualifier Setup														
🗶 Calibration Curve Setup														

2 To inspect the imported retention time data, click **Method Setup Tasks > Retention Time Setup**.

You can modify data fields in blue for individual compounds.

🖪 Agilent MassHunter Quantitative Analysis - [New Method]															
File Edit View Analyze Method Update Report Tools Help															
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dhod Tasks 🗸 Kehod Table															
New / Open Method	New / Open Method Evels 10 Create Levels Time Segment:														
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New Method from Acquired Scan Data with Library Se.															
New Method trom Acquire's Scall Uata with Library Se. Commission Commiss															
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		-	Amp	1	136.2 -> 91.	4	MRM		Tar	rget	2.102	1.000	1.000	Minutes	
Upen Method from Existing Batch			Amp-d5	1	141.1 -> 93.	4	MRM		IST	rd .	2.078	1.000	1.000	Minutes	
Method Setup Tasks			Cocaine	1	304.1 -> 18	2.0	MRM		Tar	rget	2.449	1.000	1.000	Minutes	
		2.00	Cocaine-d3	1	307.1 -> 18	5.0	MRM		IST	ſD	2.450	1.000	1.000	Minutes	
MRM Compound Setup			MDMA	1	194.2 -> 16	3.3	MRM		Tar	rget	2.269	1.000	1.000	Minutes	
/ Retention Time Setup			MDMA-d5	1	199.2 -> 16	1.3	MRM		IST	ſD	2.269	1.000	1.000	Minutes	
-Autorn o			Meth	1	150.1 -> 11	9.3	MRM		Tar	rget	2.239	1.000	1.000	Minutes	
BY ISTU Setup		(Meth-d5	1	155.2 -> 92.	3	MRM		IST	TD	2.233	1.000	1.000	Minutes	
n Concentration Setup															
T Qualifier Setup															

Task 3. Set Up Target Compounds

- **3** Assign the corresponding deuterated compound as the internal standard (ISTD) for each target compound.
 - a Click Method Setup Tasks > ISTD Setup.
 - b For each target compound row, click the down arrow in the ISTD
 Compound Name cell. Do not attempt to enter the ISTD name into the ISTD compound row.

Agilent MassHunter Quantitative Analysis - [New Meth	od]												
File Edit View Analyze Method Update Report T	ools	Help											
🛅 🗁 🛃 🗈 💭 Analyze Batch 🛛 🧭 🕴 Layout			X 💷 🕰 🗵	Restore Defa	ult Layout								
Method Tasks 🗸 🗸	Metho	od Tal	ble										
New / Open Method	i L	evel l	Name Prefix:		# of Levels: 10		Create Lev	els 🕴 Time Segmen	: 🗰 <all></all>		- 🔹 Cor	mpound: 🔙	Amp 👻 🗉
New Method from Acquired MRM Data	S	Sample	e										
New Method from Acquired Scan Data			Name	Data File	Тур	•	Level	Acq. Method File	Acq. Date-Time				
New Method from Acquired Scan Data with Library Se	[C	MAMCal_L5.d	CMAMCal_L5.	d								
New Method using Manual Setup		Qu	antifier										
Open Method from Existing File			Name	TS	Transition		Scan	Туре	ISTD Compound	Vame	ISTD Flag	ISTD Conc.	Time Reference Flag
Once Method from Existing Patels		•	Amp	1	136.2 -> 91.4	MRM		Target	Amp-d5	-			
Open Metrica from Existing Batch			Amp-d5	1	141.1 -> 93.4	MRM		Taraat	<none></none>				
Method Setup Tasks		-	Cocaine-d3	1	307.1-> 185.0	MRM		ISTD	<none></none>				
K MRM Compound Setup			MDMA	1	194.2 -> 163.3	MRM		Target	<none></none>				
Retention Time Setup.		····	MDMA-d5	1	199.2 -> 164.3	MRM		ISTD	<none></none>		V		
			Meth	1	150.1 -> 119.3	MRM		Target	<none></none>				
ISI D Setup		·	Meth-d5	1	155.2 -> 92.3	MRM		ISTD	<none></none>		1		
Concentration Setup													
X Qualifier Setup													
🛠 Calibration Curve Setup													

- c Click the ISTD name associated with the target compound.
- **d** Type the ISTD concentration (**ISTD Conc.**) for each ISTD compound (50.0000 in this example).

Agilent MassHunter Quantitative Analysis - [New Meth	hod]											
File Edit View Analyze Method Update Report	Tools	Hel	р									
🐚 🗁 🛃 📭 🖓 Analyze Batch 🛛 🥑 🕴 Layour			8 💷 🗛 🗵	Restore Defa	ault Layout							
Method Tasks 🗸 🗸	Me	thod 1	able									
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New Method from Acquired MRM Data		Sam	ple									
New Method from Acquired Scan Data			Name	Data File	Туре		Level	Acq. Method File	Acq. Date-Time			
New Method from Acquired Scan Data with Library Se	-		CMAMCal_L5.d	CMAMCal_L5	d							
New Method using Manual Setup		(Quantifier									
Doen Method from Existing File			Name	TS	Transition		Scan	Туре	ISTD Compound Nam	ne ISTD Flag	ISTD Conc.	Time Reference Fla
Open Method from Existing Batch			Amp Amp	1	136.2 -> 91.4	MRM		Target	Amp-d5		50,0000	
		-	Amp-do Cocaine	1	304 1 -> 182 0	MRM		Target	<ivone> Cocaine-d3</ivone>		50.0000	
Method Setup Tasks			Cocaine-d3	1	307.1 -> 185.0	MRM		ISTD	<none></none>	v	50.0000	
K MRM Compound Setup			MDMA	1	194.2 -> 163.3	MRM		Target	MDMA-d5			
			MDMA-d5	1	199.2 -> 164.3	MRM		ISTD	<none></none>		50.0000	
1STD Setun		-	Meth Meth-d5	1	150.1 -> 119.3	MRM		larget	Meth-db		50,0000	
Concentration Setup			Mear-do		100.2 -9 02.0	MILIM		1510	CINONES		50.0000	

2 Exercise 1: Set Up and Quantitate a Batch of Acquired MRM Data Files Task 4. Set Up Quantitation

Task 4. Set Up Quantitation

This task presents instructions for setting up the quantitation parameters for the method's:

- Calibration levels
- Qualifier ions
- Calibration curve fit
- 1 From the main menu select **Method > Create Levels from Calibration Samples**.

The Calibration table opens under each Quantifier in the Method Table.

- 2 For one of the Quantifiers, change the default concentrations to the actual concentration for each level.
 - L1-2.5000
 - L2-5.0000
 - L3-12.5000
 - L4-25.0000
 - L5-125.0000

Agilent MassHunter Quantitat	tive Anal	lysis -	New Me	thod]												
File Edit View Analyze Met	thod U	Ipdate	Report	Tools	Help											
🔁 🗁 📕 🖬 💭 Analyz	e Batch	- 6) i La	yout: 큤			📝 Rest	ore Det	fault Layo	ut						
Method Tasks 🚽 🗙	Metho	d Table														
New / Open Method	Ti	me Sec	ament:	🖛 🛛 🖛		-	Comp	ound:	😸 Amp		👻 🛋 🛛 Reset T	Table Vie	w			
Method Setup Tasks	Sa	mple		-		-							_			
K MBM Compound Setup		N	lame		Data File		Туре		1	Level	Acq. Method File	Acq.	Date-Time			
Detention Time Colum	-	CMAJ	MCal_L5	d CM	AMCal_L5.	d										
AL netendon rine setup		Quant	tifier												1	
is ISTD Setup			Name		TS	Tra	nsition		Scan		Туре		Units	Dil. Hi	h Conc.	
🦃 Concentration Setup		A	mp		1	136.2 -:	91.4	MRM			Target	ng/ml				
🛣 Qualifier Setup		C	alibration													
🚀 Calibration Curve Setup			Level	Conc.	Response	Enable	Туре		Cal. Path	Calibrat	tion STD Acquisition D	ateTime	Level Last Up	date Time	Level RSD	RF
Slobalt Satur			L1	1.0000			Calibratio	n	n				6/17/2014 3:09	PM		
	1		L2	2.0000		1	Calibratio	n					6/17/2014 3:05	PM		
Save / Exit			L3	3.0000		V	Calibratio	n					6/17/2014 3:05	PM		
🔐 Validate			L4	4.0000		1	Calibratio	n					6/17/2014 3:05	PM		
-			LD	5.0000			Calibratio	n		-			6/17/2014 3:05	PM	-	
👼 Save			14	4 0000			QC	-		-			6/17/2014 3:05	PM		+
Save As		Quant	tifier	4.0000			de						0.112014 0.00	/ 14		
🔀 Exit		Quan	Name	1	TS	Tra	nsition	Ľ.	Scan	1	Type	T	Units	Dil, Hi	ah Conc.	
Manual Setup Tasks		A	mp-d5		1	141.1 -:	93.4	MRM			ISTD	ng/ml				
Outline Colum Tanks		C	alibration													
Ouclier Secup Tasks			level	Conc	Response	Enable	Type		Cal Path	Calibrat	tion STD Acquisition D	ateTime	Level Last Up	date Time	Level RSD	RE
Advanced Tasks			111	50 0000			Calibratio	0					6/17/2014 3:05	PM		
			L2	50.0000			Calibratio	n					6/17/2014 3:05	PM		\square
			L3	50.0000			Calibratio	n					6/17/2014 3:09	PM		
			L4	50.0000			Calibratio	n					6/17/2014 3:09	PM		

3 Click Method > Copy Calibration Levels To....

Task 4. Set Up Quantitation

The system displays the Copy Calibration Levels To dialog box.

- Copy Calibration Levels To ? 🔀 Select Compounds: TS RT Transition ISTD Flag Cmpd. Group Name Meth Ш Þ Select All OK Cancel
- 4 Click Select All, and then click OK.

5 Close the **Compound Information** window and the **Sample Information** window in the lower half of the Quantitative Analysis main view.

Task 4. Set Up Quantitation

6 Browse the **Method Table** to compare the calibration concentration setup among the four target compounds, Amp, Cocaine, Meth, and MDMA.



Task 4. Set Up Quantitation

7 Under Method Setup Tasks, click Qualifier Setup, and inspect the Qualifier setup parameters.

The system automatically populates the qualifier setup parameters when it imports MRM acquisition information.

During method creation, additional MRM transitions besides the quantifier ion for a compound are assigned as qualifier ions.



8 Under Method Setup Tasks, click Calibration Curve Setup.

Task 4. Set Up Quantitation

9 For each target compound, change the **CF Origin** to **Force**.

Agilent MassHunter Quantitative Analysis - [New Meth	od]															
File Edit View Analyze Method Update Report Tools Help																
🛅 🗁 🛃 📭 💭 Analyze Batch 🛛 🕢 🔛 Layout:			🗷 💷 🖂 🗵	Restore Defa	ault Layout											
Method Tasks 👻 🗙	Met	hod T	able													
New / Open Method		Level	Name Prefix: L		# of Levels:	5		Create Lev	els	Time Segment	: 🖛 <all></all>	Ŧ	-	Compour	nd:	🖛 Meth
New Method from Acquired MRM Data		Samp	ble						_							
New Method from Acquired Scan Data			Name	Data File		Туре		Level		Acq. Method File	Acq. Date-Time					
New Method from Acquired Scan Data with Library Se		(CMAMCal_L5.d	CMAMCal_L5	d											
New Method using Manual Setup		G	luantifier													
I Onen Method from Existing File			Name	TS	Transitio	m		Scan		Туре	CF		С	F Origin		CF Weight
Coper Mealed from Existing File		-	Amp	1	136.2 -> 91.4	M	RM		Tar	get	Linear	Fo	rce		N	lone
Open Method from Existing Batch			Amp-d5	1	141.1 -> 93.4	M	RM		IST	D		_				
Method Setup Tasks			Cocaine	1	304.1 -> 182	.0 MI	RM		Tar	get	Linear	Fo	rce		N	lone
M NDN Commit School			Cocaine-d3		307.1 -> 185	0 Mi	RM		151	U	Parasa.				┥.	
AL MRM Compound Setup		-	MDMA JE		194.2 -> 163	3 MI			Iar	get	Linear	10	rce		- "	ione
K Retention Time Setup			Meth	1	150.1-> 119	3 MI	RM		Tar	oet.	Linear	Eo	rce		- 1	lone
if ISTD Setup			Meth-d5	1	155.2 -> 92.3	MI MI	RM		IST	D	Lindar					
2 Concentration Setup																

10 Under Save/Exit, click Validate to validate the method setup. You can view any validation errors that do occur at the bottom of the screen.

Agilent MassHunter Quantitative Analysis - [New Meth	od]									
File Edit View Analyze Method Update Report To	ools Hel	p								
🐚 📴 📕 📭 🖓 Analyze Batch 🛛 🕢 🛛 Layout:	12 III	81 III 🗛 🖻	Restore Defau	It Layout						
Method Tasks 👻 🗙	Method T	able								
New / Open Method	Leve	I Name Prefix: L		F of Levels: 5		Create Lev	els Time Segment	: 🗰 <all></all>	👻 📫 🛛 Compoun	d: 💷 Meth 👻 🗉
New Method from Acquired MRM Data	Sam	ple								
New Method from Acquired Scan Data	Name Data		Data File	Type		Level	Acq. Method File	Acq. Date-Time		
New Method from Acquired Scan Data with Library Se		CMAMCal_L5.d	CMAMCal_L5.d	i.d						
New Method using Manual Setup	(Quantifier								
(A Onen Method from Evistion File		Name	TS	Transition	5	ican	Type	CF	CF Origin	CF Weight
		Amp	1	136.2 -> 91.4	MRM		Target	Linear	Force	None
Open Method from Existing Batch		Amp-d5	1	141.1 -> 93.4	MRM		ISTD	12	F	New
Method Setup Tasks		Cocaine Cocaine	-	304.1 -> 182.0	MDM		I arget	Linear	Force	INONE
K MRM Compound Setup		MDMA	1	194.2 -> 163.3	MRM		Target	Linear	Force	None
K Retention Time Setup	1 19	MDMA-d5	1	199.2 -> 164.3	MRM		ISTD			
uter ISTD Satur	1 14	 Meth 	1	150.1 -> 119.3	MRM		Target	Linear	Force	None
Man and an	1 1	Politi-00		100.2 - 2 02.0	[MINRO		1510			
Concentration Setup			(A.	land Manual London	. 0	. An abusia	2	1		
X Qualifier Setup			Ag	ient masshunce	a Quantitati	re Analysis	- 65			
🛠 Calibration Curve Setup				_						
I Globals Setup				Method	d validated. I	No errors or v	varnings found.			
Save / Exit										
Validate							01			
Ma Save							UK			
Save As			_					-		
K Exit										

- 11 After the validation message appears, click OK.
- 12 Click Save/Exit > Exit.
- **13** Select **None** under **Additional batch processing after applying the method**, and click **Yes** to the **Would you like to apply this method to the batch?** prompt.

-				
Wo	ild you like to apply this n	ethod to the batch?	1	
	Yes	No	Cancel	
Additional bate	h processing after applyi	ng the method	_	_
C Analyz	e 🗇 Quantitate	D Integra	te 🔍 N	one

2 Exercise 1: Set Up and Quantitate a Batch of Acquired MRM Data Files Task 5. Set the Integrator

Task 5. Set the Integrator

Step 1 Change the default integrator to MS-MS

The default and Agilent recommended integrator for MassHunter Quant is the Agile2 parameterless integrator. This task changes the default Agile2 integrator to the MS-MS integrator to demonstrate the procedure for changing the integrator for all compounds in a Quant method.

- 1 Click Method >Edit or press F10.
- 2 Click Method > Advanced Tasks > Integration Parameters Setup.
- 3 In the Method Table, click the box located on the right side of the Int. value.

Met	thoo	Tal	ble											
1	Tir	ne S	Segmer	nt: 🖛	<a< th=""><th> ></th><th></th><th>-</th><th></th><th>Compound: [</th><th>🖨 Am</th><th>ιр</th><th>÷</th><th>E</th></a<>	>		-		Compound: [🖨 Am	ιр	÷	E
	Sa	mple	e				~							
		Name		Data File Type		Туре	Level	Acq. Method File		Acq. Date-Time				
		Ca	alib-L5	CMAN	(Cal	_L5.d	Cal	L5	AP	Clautotune.m	5/12/2	006 2:03 F	PM	
		Qu	antifier											
			Nam	e	TS	Tra	ansition	Sc	an	Туре	RT	Int.	Int. P	arm
	÷		▶ Amp		1 136.2 ->		-> 91.4	> 91.4 MRM		Target	2.102 Agile2			
			Qualif	ier			an an air an t-s							

Task 5. Set the Integrator

- Integration
- 4 Select **MS-MS** from the drop-down menu.

- 5 Click Apply to All.
- 6 Click OK.
- 7 Under Save/Exit, click Exit.
- 8 Select None under Additional batch processing after applying the method, and click Yes to the Would you like to apply this method to the batch? prompt.

2 Exercise 1: Set Up and Quantitate a Batch of Acquired MRM Data Files Task 6. Analyze and Save the Batch

Task 6. Analyze and Save the Batch

In this exercise, you quantitate the batch and then save the results.

- 1 Click the **Analyze Batch** icon [Analyze Batch] in the toolbar to start batch analysis.
- **2** Pass the cursor over the quantitation message for Sample 1.
- **3** Pass the cursor over the flags for the first two calibration standards. Note that two calibration standards contain outlier data

Note that the program found no data for Amphetamine (Amp) in Sample-1.

	-	Samp	ole:	Calib-L5	5	- 1	Sam	ple Type: <all></all>	
					Sam	ple			Amp Met
		•	8	Name	Data File	Туре	Level	Acq. Date-Time	Exp. Conc.
		0	٣	Blank-1	CMAMBIk_01.d	Blank		5/12/2006 1:48 PM	
Outlier flag			٣	Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	2.5000
messages			٣	Calib-L2	CMAMCal_L2.d	Cal	L2	5/12/2006 1:54 PM	5.0000
				Calib-L3	CMAMCal_L3.d	Cal	L3	5/12/2006 1:57 PM	12.5000
		1 × 1		Calib-L4	CMAMCal_L4.d	Cal	L4	5/12/2006 2:00 PM	25.0000
	•			Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM	125.0000
Quantitation		· · · · · ·		QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM	5.0000
message				QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	25.0000
		0	٣	Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM	
				Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM	
				Sample-3	CMAMSam_03.d	Sample		5/12/2006 2:18 PM	
							- 10 - 11	8	

- 4 Click File > Save Batch.
- 5 Click File > Close Batch to close the batch.

2 Exercise 1: Set Up and Quantitate a Batch of Acquired MRM Data Files Task 6. Analyze and Save the Batch

Task 1. Set Up a New Batch 27

Task 2. Set Up a New Method for the Batch 30

Task 3. Set Up Target Compounds 34

Task 4. Set Up Quantitation 35

Task 5. Analyze and Save the Batch 37

In this exercise, you set up a quantitation method for a batch of acquired Q-TOF data files. You carry out the exercise with the **LC-QTOF Pesticide** data files on your installation media and learn how to perform the following tasks:

- Set up a Batch Table containing sample and calibration data files for the solvent.
- Set up a new quantitation method based on the calibration standard of the highest concentration.
- Set up a target compound.
 - View the product ion and chromatographic parameters for the solvent compound in the data file.
- Set up quantitation for the method.
 - Create levels from calibration samples.
 - Set up qualifier ions and the calibration curve.
- Quantitate the batch and save the results.

Before you begin...

Make sure that you have copied the **LC-QTOF Pesticide** folder from the **Supplemental/Data/Quant Examples/Q-TOF** folder of the installation media to a folder on your system. If the default MassHunter Quantitative Analysis Software Supplemental installation has been completed, then the data files needed for these exercises should be present in **MassHunter/Data/QuantExamples**.

3 Exercise 2: Set Up and Quantitate a Batch of Acquired Q-TOF Data Files Task 1. Set Up a New Batch

Task 1. Set Up a New Batch

In this task, you set up a Batch Table containing data files for calibration samples of the solvent. Many of the tasks in this section are similar to the tasks in Exercise 1.

1 To start the Quantitative Analysis program, click the Quantitative Analysis (Q-TOF) icon on your Desktop . When you first use the program, the default layout appears, as shown in Figure 4.

You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (Q-TOF)** from the Start menu.





2 Click File > New Batch.

The system opens the New Batch dialog box.

- 3 Navigate to the folder \Your Directory\LC-QTOF Pesticide\.
- 4 Type the batch file name *iii_Test_01* and click Create.

3 Exercise 2: Set Up and Quantitate a Batch of Acquired Q-TOF Data Files Task 1. Set Up a New Batch

If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new batch.

The system displays the **Add Samples** dialog box. **All Samples** should be selected.

5 Click **OK** to add them to the batch.

The Batch Table is no longer empty. It now contains the samples. See **Figure 5** on page 29.

• Note that there are four calibration samples.

Add Samples	? ×
Batch Folder: D:\Ma	ssHunter\Data\Qua
File name	Sample name
Leek_Spike_5.d	Leek_100 ppb
Pepper_Spike_5.d	Pepper_100 ppb
Solvent_Cal_4.d	Solvent_5 ppb
Solvent_Cal_6.d	Solvent_20 ppb
Solvent_Cal_7.d	Solvent_50 ppb
Solvent_Cal_8.d	Solvent_100 ppb
<	-
Browse to Copy Samp	oles
Translate MSWS Sam	ples
Select All OK	Cancel

Exercise 2: Set Up and Quantitate a Batch of Acquired Q-TOF Data Files Task 1. Set Up a New Batch 3

1 P	7 4	Anal La Anal	vze Batch 🕜 :	Lavout:			estore De					
. T.				cojoca 🕰								
IId	Die											
amp	ole:	<u>•</u>	Sample Typ	e: <all></all>	Ŧ	Compound: 🔙				*		STD:
			Sample									
1	7	Name	Data File	Туре	Level	Acq. Date-Time						
1		Solvent 5 ppb	Solvent Cal 4.d	Cal	1	4/14/2011 8:53 PM						
1	1	Solvent 20 ppb	Solvent Cal 6.d	Cal	2	4/14/2011 9:26 PM						
1	1	Solvent_50 ppb	Solvent_Cal_7.d	Cal	3	4/14/2011 9:43 PM						
1		Solvent_100 ppb	Solvent_Cal_8.d	Cal	4	4/14/2011 9:59 PM						
1		Pepper_100 ppb	Pepper_Spike_5.d	Sample		4/15/2011 9:45 PM						
		Leek_100 ppb	Leek_Spike_5.d	Sample	1	4/16/2011 5:28 AM						
our	nd Info	ormation				• X	Calibra	ion Cur	/e			
our	nd Info	ormation		孟山金		• X	Calibra	ion Cur	/e		Туре	21
our •	nd Info + 1 ogran	ormation		َه لل <u>⊼</u> [±	- × ×	Calibra	ion Cur ↔ ‡	/e		Туре	2
our Anat	nd Info	ormation C A I I I	5 0 0 <u>A</u>	à III 五	£ _	- X	Calibra : v	ion Cur ↔ ‡ 2]	/e	æ 🕸	Туре	2
our • nat 0 1	nd Info	ormation	J @ @ <u>A</u>	≩ بلا <u>⊼</u>	2 4	- X	Calibra	ion Cur ↔ ‡	/e		Туре	21
our • • 0 1 0.8 0 7	ogran	ormation		≩ بلا <u>⊼</u>	<u></u>	• X	Calibra Sa x10 Sa x10	ion Cur ↔	/e		Туре	22
our nat 0 1 0.8 0.7	nd Info	ormation		<u>که ال کر</u>	± "A.	• X	Calibra Sas ×10 Sas ×10 Calibra	ion Cur ↔ ‡ 2 	/e		Туре	21
our at 0 1 0.8 0.7 0.6 0.6	nd Info	ormation		≩ u ∑	<u></u>	- X	Calibra Sa ×10 Sa ×10 Calibra Sa ×10 Calibra Sa ×10 Calibra Sa ×10 Calibra	ion Cur ↔ 2 	/e		Туре	
our at 0 1 0.8 0.7 0.6 0.5	nd Info	ormation		<u>ه</u> ال ۲	<u></u>	- X	Calibra Sa x10 Sa x10 Sa x10 O O O	ion Cur ↔ 2 - - - - - - - - -	/e		Туре	22
our 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1	ogran	ormation		<u>Т</u> Ш £	<u></u>	× X	Calibra 	ion Cur	/e		Туре	22
our nat 0.8 0.7 0.6 0.5 0.4 0.3	nd Infe	n		<u>ه</u> الا ۲ <u>ـ</u>	<u></u>	• X	Calibra 	ion Cur ↔ 2 - - - - - - - - - - - - -	/e		Туре	22
our 0 1 0 1 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.2	ogran	ormation		五 山 重	2	- X	Calibra 	ion Cur ↔ 2 	/e		Туре	

Batch Table containing samples before quantitation Figure 5.

3 Exercise 2: Set Up and Quantitate a Batch of Acquired Q-TOF Data Files Task 2. Set Up a New Method for the Batch

Task 2. Set Up a New Method for the Batch

This task shows you how to set up a new quantitation method based on a batch containing calibration sample data files. In this task we will use a single calibration sample and extract from it the necessary data to add a calibration compound to the method.

The procedure described in Task 2 is a manual one. There is also an automated procedure in MassHunter that allows you to create a quantitation method that adds a large number of calibration compounds in a single step using acquired scan data with a library search. In the automated method, MassHunter analyzes a data file, and using search ID parameters that you specify, identifies compound names, the target ion, qualifier ions and ratios, and retention times. Then it uses this information along with other default parameters to fill in initial values for the quantitation method. This automated method greatly reduces the time required for method creation.

Additionally, you can add compounds found in Qualitative Data Analyses by transferring the data from Qual to Quant using CEF files. Refer to your online Help for more details.

1 Use the cursor to highlight the calibration standard that has the highest concentration level, as shown in the figure below.

Using a sample with strong signals for the compounds, such as a high-concentration calibration sample, lets the program create a method with the appropriate retention times and qualifier ratios.

Batch Ta	able					2	
Sam	ple:	Solvent 🔻	Sample Typ	e: <mark><all></all></mark>	-	Compound: 💓	
			Sample				
•	8	Name	Data File	Туре	Level	Acq. Date-Time	
		Solvent_5 ppb	Solvent_Cal_4.d	Cal	1	4/14/2011 8:53 PM	
		Solvent_20 ppb	Solvent_Cal_6.d	Cal	2	4/14/2011 9:26 PM	
		Solvent_50 ppb	Solvent_Cal_7.d	Cal	3	4/14/2011 9:43 PM	
•		Solvent_100 ppb	Solvent_Cal_8.d	Cal	4	4/14/2011 9:59 PM	
		Pepper_100 ppb	Pepper_Spike_5.d	Sample		4/15/2011 9:45 PM	
		Leek_100 ppb	Leek_Spike_5.d	Sample	3	4/16/2011 5:28 AM	

Task 2. Set Up a New Method for the Batch

2 Click **Method > Edit** to switch to method editing mode.

The **Method Tasks** appear in the column to the left of the View, as shown in **Figure 6** on page 31.

Note that Figure 6 shows the default layout for method editing.

3 If the default layout is not present, click **Restore Default Layout** on the toolbar before creating a new method in the next step.





4 In the **Sample Information** window, click the middle of the peak at approximately 4.82 on the x-axis. Right-click and click **Extract Spectrum**.

Task 2. Set Up a New Method for the Batch

5 Click the largest ion, **396.0966**. Right-click that location and click **New Compound**.

To accurately select the ion, hold down the right mouse button while hovering over the spectra and zoom in on the range around the ion you are trying to select.

Cualifier Setup		
Calibration Curve Setup	Sample Information	
🖉 Globals Setup	🛃 ↔ ‡ 🔍 🔍 🐐 Max # of panes: 2 🚽 📊 🔐	
Save / Exit	+ TIC Scan (** -> **) Solvent Cal 8.d	
🞯 Validate	₩ ×107 = ×107 3-	
📰 Save	о ² -	
Save As	1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1	h
X Exit	0	4.8 4.9 5 5.1 5.2 5.3 5.4 5.5 5
Manual Setup Tasks		Acquisit
Outlier Setup Tasks	+ Scan (4.804-4.804 min, 1 scans) Solvent_Cal_8.d	
Advanced Tasks	5 0.8-	Copy Ctrl+C
	0.6-	🛃 Auto Scale
	0.4	\leftrightarrow X - Auto Scale
	418.0772	Y - Auto Scale
		Revious Zoom
	175.00 200.00 225.00 250.00 275.00 300.00 325.00 350.00 375.00 400.00 425.00 4	🔍 Next Zoom
		New Compound
	Compound Information	New Qualifier
	❷ ↔ ‡ 丞 盂 ⑧ ⑧ ◎ ◎ ▲ ▲	🔎 Search Library
	Chromatogram	Search Library Settings

- 6 Type Tribenuron-methyl as the Name in the Method Table. Keep this compound selected in the Method table while you add the qualifier in the next step.
- 7 To once again display the spectrum for **Tribenuron-methyl**, click at the peak apex to display a line running through the apex.

Task 2. Set Up a New Method for the Batch

8 Click **418.0776** to select that ion (blue filled triangle). Right-click that location and click **New Qualifier**.

You can select more than one qualifier ion.

A blue triangle indicates the selected m/z in the spectrum. The qualifier is added to the Method Table as shown.



3 Exercise 2: Set Up and Quantitate a Batch of Acquired Q-TOF Data Files Task 3. Set Up Target Compounds

Task 3. Set Up Target Compounds

With this task, you learn to inspect the product ions and the RT data for the new quantitation method, which you can change for individual target compounds. Check the new quantitation method created from the **Sample Information** window for the product ion:

- 1 To inspect the retention time set from the spectrum, click **Method Setup Tasks > Retention Time Setup**.
- 2 In the Left RT Delta column, enter 0.2.
- 3 In the **Right RT Delta** column, enter 0.2.

You can modify data fields in blue for individual compounds.

mple											
Name Data File		е Тур	e	Level		Acq. Method File	Acq. Date-Tin	ne -			
Solvent_100 ppb	Solvent_Cal	8.d Cal		4		TG_Pesticides	4/14/2011 9:59	L			
Quantifier											
Name	TS	Transition	Scan		Туре		RT	Left RT Delta	Right RT Delta	RT Delta Units	
Tribenuron-me	eth	1 396.0966	Scan		Tai	rget	4.813	0.200	0.200	Minutes	

11.11.17.1

3 Exercise 2: Set Up and Quantitate a Batch of Acquired Q-TOF Data Files Task 4. Set Up Quantitation

Task 4. Set Up Quantitation

This task presents instructions for setting up the quantitation parameters for the method's:

- Calibration levels
- Qualifier ions
- Calibration curve fit
- From the main menu select
 Method > Create Levels from Calibration Samples.

The Calibration table opens under each Quantifier in the Method Table.

- **2** For one of the Quantifiers, change the default concentrations to the actual concentration for each level.
 - L1-2.5000
 - L2-20.0000
 - L3-50.0000
 - L4-100.0000

mp	le									
Name /			Data File		Level	Acq. Method File		Acq. Da	ate-Time	
Solvent_100 ppb Solv		ent_Cal_8.d	Cal	4	TG_Pesticides_TOF	_Pesticides_TOF_3Hz_DD.m		:59 PM		
Q	uantifier									
	Name	1	TS	Trans	sition	Scan		Туре	Units	-
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	2		20	0.0000		V				
	- 3		50	0000.						
	4		100	0000.0						

Task 4. Set Up Quantitation

3 Click Save/Exit > Validate to validate the method setup.

You can view any validation errors that do occur at the bottom of the screen.

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D Ph P P P T Analyze method opd	
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New / Open Method	Time Segment de CAlls y de Compound de y de Recettable View
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E and a comp	
Save / LAR	+ 11C Sean ("->") Souvent_cal_8.d x10.7"
igg validate	27 26 23 3 31 32 33 34 33 36 37 36 33 4 4.1 42 43 44 45 46 47 46 43 5 51 152 53 54 50 55 50 57 56
Re Save	Scan (4.809-4.809 min, 1 scans) Solvent_Cal_8.d Solvent_Cal_8.d Solvent_Cal_8.d Solvent_Cal_8.d
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Manual Setup Tasks	Mass-to-Charge (m/z)
Outlier Setup Tasks	Compound Information Agulent MassHunter Quantitative Analysis
Advanced Tasks	
	Chromatogram Ul Method validated. No errors or warnings found.
	08
	02-
	U-1
	Method Error List 🗸 🗸 🗸
	Category Message
	1 Compounds (1 total) 0 ISTD (0 total)

- 4 After the validation message appears, click **OK**.
- 5 Under Save/Exit, click Exit, then select None under Additional batch processing after applying the method, and click Yes to the Would you like to apply this method to the batch? prompt.
3 Exercise 2: Set Up and Quantitate a Batch of Acquired Q-TOF Data Files Task 5. Analyze and Save the Batch

Task 5. Analyze and Save the Batch

In this exercise, you automatically quantitate the batch and then save the results.

1 Click the **Analyze Batch** icon **[]** <u>Analyze Batch</u> in the toolbar to start batch analysis.



- 2 Click File > Save Batch.
- 3 Click File > Close Batch to close the batch.

3 Exercise 2: Set Up and Quantitate a Batch of Acquired Q-TOF Data Files Task 5. Analyze and Save the Batch

Task 1. Navigate the Batch Table Results 40

Task 2. Change Result Window Layouts 44

Task 3. Export and Print Results 50

The tasks in this exercise show you how to inspect the sample and compound data in a batch file, customize result layouts, export your data to Microsoft Excel, and preview and print the data.

Use the **DrugsOfAbuse** batch in this exercise. Similar tasks can be performed with Triple Quad data files, Q-TOF data files, and TOF data files.

Task 1. Navigate the Batch Table Results

Task 1. Navigate the Batch Table Results

This task shows you how to scroll through your samples and compounds, while observing changes in the Batch Table and compound information data. It also shows you how to display various sample types.

Step 1 Open the batch file *iii*_Test_01.batch.bin, created in Exercise 1.

- 1 To start the Quantitative Analysis program, click the **Quantitative Analysis** icon on your Desktop.
- 2 Click **Open Batch** in the toolbar to display the **Open Batch** dialog box.
- 3 Navigate to \Your Directory\DrugsOfAbuse and click iii_Test_01.batch.bin

The main view that appears should look like the one below. This is the default layout and contains the default column settings.

	Agile	nt M	assHunter (Quantitative Analy	sis - DrugsO	fAbuse	_80500 - iiii_Test_01													• 🗙
Fi	le E	dit '	View Anal	yze Method Up	odate Repo	rt Tool	s Help													
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•	0		Blank-1	CMAMBIk_01.d	Blank		5/12/2006 1:48 PM			MS-MS										
	-	<u><u></u></u>	Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	2.5000		MS-MS	2.141	658		3.3187	3.3187	132.7	24.3	2.1	29 1397	25.9
	-	۲	Calib-L2	CMAMCal_L2.d	Cal	12	5/12/2006 1:54 PM	12,6000	(m)	MS-MS MC MC	2.140	1059	100	5./493	5.7493	109.4	33.5	2.1	28 1298	25.9
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	-		Calib-L4	CMAMCal 15 d	Cal	15	5/12/2006 2:03 PM	125,0000		MS-MS	2 101	18605	100	124 4844	124 4844	99.6	27.0	20	76 1053	26.4
			QC-L2	CMAMQC L2.d	QC	L2	5/12/2006 2:06 PM	5.0000		MS-MS	2.142	1006		5.2293	5.2293	104.6	27.7	2.1	31 1356	31.1
			QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	25.0000		MS-MS	2.135	4716		27.8039	27.8039	111.2	25.6	2.1	21 1196	31.1
	0		Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM			MS-MS										
			Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM			MS-MS	2.143	1004		4.8977	4.8977		30.9	2.1	30 1445	25.7
			Sample-3	CMAMSam_03.d	Sample		5/12/2006 2:18 PM			MS-MS	2.105	2590		14.2183	14.2183		25.3	2.0	39 1284	29.8
Counts +	■ ↔ RM.f1 1- 0.9- 0.8- 0.7- 0.6- 0.5- 0.4- 0.3- 0.2- 0.1- 0-2- 0.1-	36.2	>> 91.4) CM	AMBIK_01.d		21		27 28 2		Amp - 5 Le ss x10 1 - 1.6- 0.8- 0.6- 0.4- 0.2- 0-	* = 7.093 R^2 = 0.5 Type:Lin	tyrels Use 492 * x 19942480 ear. Orig	ed, 5	Type: Li 5 Points, 5 Poi orce. Weight 1	Origin: Ints Used. 2 C None		Weight	N		
		_	a 1.3 1.	e 1.0 1.0 1.7			Acqui	isition Time (min)		U	0.2	0.4	0.0 0.8	1 1.2	1.4	1.0 1.	°ŕ	elative C	oncentration
												Pro	ces	sed Blan	k-1)	Amp	11	Samples	(11 total)	TW/cm

Task 1. Navigate the Batch Table Results

Step 2 (Optional) If you see a different layout than the one in the figure on the previous page...

- If fewer than three windows are present in the main view, or they are in a different arrangement, restore the default layout.
- If the column settings are different, restore the default column settings.
- If panes other than the Chromatogram pane are present in the **Compound Information** window, hide the other panes.
- To restore the default layout, click Restore Default Layout on the toolbar before scrolling from sample to sample.
- To restore the default column settings, right-click anywhere in the **Batch Table** window and click **Restore Default Columns**.
- To hide extra panes, click the highlighted icons other than the Show/Hide Chromatogram icon 🛆 in the Compound Information toolbar.
- The default layout is set at the factory and cannot be changed. If you want to create your own layout, see **"Task 2. Change Result Window Layouts"** on page 44.

Step 3 Scroll from sample to sample until you reach the end of the Batch Table, and then return to Cal-L5.

- 1 Click the **Next Sample** arrow 1 in the Batch Table Standard toolbar until the system displays the desired sample. Inspect the changes in the **Compound Information** window.
- 2 To return to Cal-L5, click the **Previous Sample** icon 1 in the Batch Table Standard toolbar.
- 3 Select any cell in the row for sample **Calib_L4** in the **Batch Table** window to view the changes.

Note the linkage between the highlighted data file in the **Batch Table** and the chromatogram in the **Compound Information** window.

Note the changes in the **Batch Table** and **Compound Information** of amphetamine for each sample.

Step 4 Scroll from compound to compound through all four compounds.

- 1 Click the Next Compound or Previous Compound arrow in the toolbar until the system displays the desired compound. Compound: 1: Meth
- 2 Inspect the changes in the **Batch Table**, **Compound Information**, and **Calibration Curve** windows.

Task 1. Navigate the Batch Table Results

- 3 Click the down arrow next to the **Compound** list.
- 4 Click Cocaine.

Step 5 Examine results for multiple compounds.

View the RT for each compound for the Cal-L4 sample.

After reviewing the results for all the compounds, return to viewing the cocaine results.

1 Click the **Display Multiple Compounds/Samples in Batch Table View** icon in the toolbar to display the quantitation results for all target compounds. You can also click **View > Batch Table Layout > Multiple Compounds/Samples View**.

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2 Click the Cal-L4 cell, and note the difference in **RT** in the **Compound Information** window for each compound.

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				Sar	nple			Coca	aine I	Method			-	Cocaine Resu	ilts		Qualif	ier	Cocaine	e-d3 (I_	Quali
		Ÿ	Name	Data File	Туре	Level	Acq. Date-Time	Exp. Conc.	IPM	Int.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	RT	Resp.	Ratio
	0	٣	Blank-1	CMAMBIk_01.d	Blank		5/12/2006 1:48 PM			MS-MS	2.433	20		11.8235	11.8235				2.403	15	
			Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	2.5000		MS-MS	2.453	5189		2.3087	2.3087	92.3	3.7		2.452	20245	4.0
			Calib-L2	CMAMCal_L2.d	Cal	L2	5/12/2006 1:54 PM	5.0000		MS-MS	2.454	9716		4.2682	4.2682	85.4	3.9		2.453	20506	4.0
-			Calib-L3	CMAMCal_L3.d	Cal	L3	5/12/2006 1:57 PM	12.5000		MS-MS	2.459	25187		11.5607	11.5607	92.5	3.9		2.459	19625	4.4
			Calib-L4	CMAMCal_L4.d	Cal	L4	5/12/2006 2:00 PM	25.0000		MS-MS	2.449	50649		25.2511	25.2511				2.448	18068	4.2
-			Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM	125.0000		MS-MS	2.440	199967		125.0768	125.0768	100.1	3.8		2.440	14401	3.7
-			QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM	5.0000		MS-MS	2.453	9246		4.2831	4.2831	85.7	3.5		2.453	19446	4.4
-			QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	25.0000		MS-MS	2.455	48582		24.5377	24.5377	98.2	4.0		2.454	17834	3.9
	0		Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM			MS-MS											
			Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM			MS-MS	2.460	9735		4.3735	4.3735		3.6		2.459	20051	3.6
			Sample-3	CMAMSam 03 d	Sample	1	5/12/2006 2·18 PM			MS-MS	2 4 4 6	24841		10 9299	10 9299		3.9		2 4 4 5	20472	3.6

Task 1. Navigate the Batch Table Results

- 3 To return to the display of detailed quantitation results for the selected target compound, click the **Display Single Compound/Sample in Batch Table** icon in the toolbar.
- 4 If necessary, click the down arrow next to the Compound list, and click Cocaine. A different set of columns is displayed when you are in Multiple Compounds/Samples View mode versus Single Compound View mode. If you add a column to the table when you are in Multiple Compounds/Samples View mode, that change is not automatically made in the Single Compound/Sample View mode.

Step 6 View selected sample types. Display only the calibration standards and then display all sample types:

1 Click the down arrow in the **Sample Type** drop down list. The **Sample Type** dialog box is displayed.

Batch Table		
Sample: 1 Calib-14 T		
	nple Type: <all></all>	ound: 🔙 🗛
Compound Group: <all></all>	Sample Type	▼ ISTD:
Sa	Al>	Am
♥ Name Data File ● Blank-1 CMAMBIk_01.d ● Calib-L1 CMAMCal_L1.d ● Calib-L2 CMAMCal_L2.d Calib-L3 CMAMCal_L3.d ● Calib-L5 CMAMCal_L4.d ● Calib-L6 CMAMCal_L3.d ● Calib-L5 CMAMCal_L4.d ● Calib-L5 CMAMCal_L5.d ● CAL4 CMAMSam_01.d ● Sample-3 CMAMSam_03.d	Sample Sample Cal CC DoubleBlank MatrixSpike MatrixSpike MatrixSpikeCup MatrixBlank TuneCheck ResponseCheck	me Exp M M M M M M M M M M M M M M M M

2 Clear the <All> check box and mark the Cal check box.

3 Click OK.

The **Batch Table** should contain only the **Cal** standards for cocaine.

- 4 Click the down arrow in the Sample Type drop down list.
- 5 Click <All>, and then click OK. The system marks all the check boxes and displays all sample types.

Task 2. Change Result Window Layouts

Task 2. Change Result Window Layouts

This task shows you how to customize your layout and how to recreate the default layout.

Step 1 Use layout icons on the toolbar to position the Batch Table, Compound Information, and Calibration Curve windows:

- 1 Click the Layout Table Left icon in the toolbar
- Click the Layout Table Right icon in the toolbar .
- 3 Click the Layout Table Top icon

Step 2 Use layout icons on the toolbar to maximize each individual window:

- 1 Click the Maximize Table icon in the toolbar
- Click the Maximize Compound Information icon in the toolbar <a>[]
- 3 Click the Maximize Calibration Curve icon in the toolbar
- **4** To return to the default layout, click the **Restore Default Layout** icon on the toolbar.

Step 3 Change the panes in the Compound Information window for Cal-L4:

- 1 In the Batch Table, select the Cal-L4 row.
- 2 In the **Compound Information** toolbar, click the **Show/Hide Qualifiers** icon
- 3 Click the Show/Hide Spectrum icon 1
- Click the Show/Hide ISTD icon <a>href="https://www.sci.action.com">sci.action.com The layout and results look like those in the following figure.

Task 2. Change Result Window Layouts

This step assumes that you started this task with just the Chromatogram pane in the **Compound Information** window.

	Agilen	t MassHunter (Quantitative Analy	sis - DrugsOf	Abuse_	80500 - iiii_Test_01												
E Fil	le Ed	it View Anal	yze Method Up	date Repor	t Tool	s Help												
-) 🗁		Analyze Batch	🕜 🕴 Lay	out: 🗔		Restore D	efau	It Layout									
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	ample	e 👔 Calib-L4	🗸 👻 🚺 San	nple Type: <	All>	Compound:	Cocaine			- U	⇒ ISIL): Cocaine-d3				14		~~
			San	nple			Coca	aine I	Method			Cocaine Res	ults		Qualifi	er Coo	caine-d3 (I	Qualifier
		V Name	Data File	Туре	Level	Acq. Date-Time	Exp. Conc.	IPM	Int.	RT	Resp.	MI Calc. Conc	Final Conc.	Accuracy	Ratio	MI R	T Resp.	Ratio M
	0	P Blank-1	CMAMBIk_01.d	Blank		5/12/2006 1:48 PM			MS-MS	2.433	20	11.823	5 11.8235			2.4	403 15	i [
	_	Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	2.5000		MS-MS	2.453	5189	2.308	7 2.3087	92.3	3.7	2.4	452 20245	4.0
		Calib-L2	CMAMCal_L2.d	Cal	12	5/12/2006 1:54 PM	5.0000		MS-MS	2.454	9/16	4.268	4.2682	85.4	3.9	2.4	453 20506	4.0
		Calib-L3			14	5/12/2006 1:57 FM	25,0000		MS-MS	2.405	20107	25 251	25 2511	101.0	3.5	2.4	148 18069	4.4
H		Calib-L5	CMAMCal L5.d	Cal	1.5	5/12/2006 2:03 PM	125,0000		MS-MS	2.448	199967	125.076	125.0768	100.1	3.8	24	448 14401	3.7
		QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM	5.0000		MS-MS	2.453	9246	4.283	4.2831	85.7	3.5	2.4	453 19446	4.4
		QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	25.0000		MS-MS	2.455	48582	24.537	24.5377	98.2	4.0	2.4	454 17834	3.9
	0	Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM			MS-MS									
		Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM			MS-MS	2.460	9735	4.373	4.3735		3.6	2.4	459 20051	3.6
		Sample-3	CMAMSam_03.d	Sample		5/12/2006 2:18 PM			MS-MS	2.446	24841	10.929	0 10.9299		3.9	2.4	445 20472	3.6
Com	pound	Information		_		_		- ×	Calibration	n Curve								÷ 3
1	• ↔	‡ <u>🥀 </u>	1		<u>ш</u>	1			i 🛃 ↔	÷ [📼 🛙	🔹 Type: Li	- Origin:	F 🔻	Weight:	N 🔻	ISTD 🗧	QC CC
+ MF	RM (30	04.1 -> 182.0) (MAMC 304.1 ->	182.0 , 304.	-> 82.0	+ MRM (2.339-2.5	i68 min, 37 s	sca	Cocaine -	5 Levels,	5 Levels	Used, 5 Points,	Points Used,	2 QCs				
Counts	10 4 - 1 - 0.5 - 0 -	2.44	min. 4 0.75 4 0.75 4 0.75 4 0.25 2.5 4 0.25 4 0	Ratio = 3.9	2.5	%) 2 ×10 5 0.75 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.	182.0 50 200 250	300	8 × 10 ¹ 	y = 5.550 R^2 = 0.1 Type:Lin	0779 1 x 99985846 lear, Origi	in:Force, Weight	None			/	/	•
A ME	DM (2)	17.1 -> 195.0) (MAMC 207.1 >	105.0 207		MPM (2 400-2 F	57 min 26 a	(1112)	0.6-					-				
× Courts	10 ³ 4- 2- 0-	2.44	mamc 30/.1> min. ↓ x10 ² ↔ 0.75 ↔ 0.75 ↔ 0.25 ↔ 0.25	Ratio = 4.2	(114.7	* MRM (2.400-2.5 %)	185.0	¢	0.4-		•	¥						
		2 Acquisition T	2.5 me (min)	2 Acquisitio	2.5 on Time	(min) 100 1 Mas	50 200 250 s-to-Charge	300 (m/z)		ġ	0.2	0.4 0.6 0.	B 1 1.2	2 1.4	1.6 1.	8 2	2.2 2 Relative Co	.4 2.6 oncentratio

Changing the layout changes only the position and visibility of the six panes. The panes in the **Compound Information** window are not affected by changing the layout.

Step 4 Save the default layout without the calibration curve:

- 5 Close the Calibration Curve window.
- 6 Click View > Window Layout > Save Layout.

The system displays the **Save Layout File** dialog box.

7 Name the layout file Batch Table plus Compound Information, and click Save.

Task 2. Change Result Window Layouts

Step 5 Load the newly created layout:

- 1 Click **Restore Default Layout** on the toolbar.
- 2 Click View > Window Layout > Load Layout.

The system displays the Load Layout dialog box.

📅 Load Layout					×
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🌗 Organize 👻 🏢 Views	; 👻 📑 New	Folder	_	_	0
Favorite Links Documents Desktop Recent Places File Computer File Pictures File Music File Recently Changed File Searches	Name	Date modified c01.d LL1.d LL2.d LL3.d LL4.d LL5.d CL2.d CL4.d LL4.d LL5.d CL4.d LL3.d LL4.d LL3.d LL4.d L	Туре	Size	
Folders	CMAMSa	m_u2.d m_03.d ults ole_Plus_Compoun	d_Informat	Layout Files (*.qu Open	antlayout xm 💌

Task 2. Change Result Window Layouts



3 Click Batch Table plus Compound Information and click Open. The results window should now look like Figure 7 on page 47.

Figure 7. Results window

Step 6 Create the layout as shown in Figure 8 on page 48:

- 1 Restore the default layout (click Restore Default Layout on the toolbar).
- 2 Right-click inside the title bar of the **Calibration Curve** window, and then mark the **Floating** check box.

Calibration Curve	
· · · ·	<u>H</u> ide
	Floating
Amn - 5 Levels 5 Low	

3 Right-click the title bar of the **Compound Information** window, and then mark the **Floating** check box.

Task 2. Change Result Window Layouts



4 Resize the windows to match the layout in **Figure 8**.

Figure 8. Display with Calibration Curve and Compound Information windows floating

5 Right-click inside the title bar of the **Compound Information** window, and then clear the **Floating** check box.

Task 2. Change Result Window Layouts



6 Resize the windows to match the layout in Figure 9.

Figure 9. Resized window

- 7 Right-click inside the title bar of the **Calibration Curve** window, and clear the **Floating** check box.
- 8 Move the **Compound Information** window so that the layout corresponds to the one pictured at the start of the task.

Step 7 Recreate (do not restore) the default layout:

- 1 Maximize the program main view.
 - Anchor the **Calibration Curve** window first, and then the **Compound Information** window, to recreate the default layout.
 - If after anchoring the two windows, the calibration curve is on the left side, right-click the title bar of the **Calibration Curve** window and drag it to the right. A gray rectangle shows where this window will be placed within the main view.
 - Drag the calibration curve to the bottom right corner of the main view.

Task 3. Export and Print Results

Task 3. Export and Print Results

This exercise shows you how to export your data to a Microsoft Excel file and how to preview and print your Batch Table and compound information data.

Step 1 Export the batch file iii_Test_01.

- 1 To make the **Batch Table** window active, click the title bar of the **Batch Table** window.
- 2 Click File > Export > Export Table.
- 3 Select My Documents as the destination directory.
- **4** Type *iii* Test 01.xlsx as the export file name.
- 5 Click Save. The Excel file My Documents\iii_Test_01.xlsx opens automatically. iii = User initials

E	🛛 Ag	ilent MassHunter Quantitative Analysis - DrugsOfAbuse_B0500 -	iiii_Test_01															
1	File	Edit View Analyze Method Update Report Tools Help		_														
1	2	New Batch	Ctrl+N	$\overline{\times}$	Restore De	efaul	t Layout											
E	0	Open Batch	Ctrl+O				_											- ×
1		Save Batch	Ctrl+S	4	Cocaine			- [ISTE): C	ocaine-d3				ę	x 🌪	7 6	$\langle \nabla \rangle$
E		Save Batch As			Coca	ine M	lethod				Cocaine Resu	ilts		Qualifi	er	Cocaine	a-d3 (I	Qualifier
		Close Batch			Exp. Conc.	IPM	Int.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	RT	Resp.	Ratio M
		Add Samples					MS-MS	2.433	20		11.8235	11.8235				2.403	15	
H		Export	•		Export Ta	able		2.453	5189		2.3087	2.3087	92.3	3.7	井	2.452	20245	4.0
H	άπ.	Page Setup			Export G	raph	ics	2.459	25187	F	4.2662	4.2602	92.5	3.9	H	2.455	19625	4.4
	A	Print	Ctrl+P		25.0000		MS-MS	2.449	50649		25.2511	25.2511	101.0	3.9		2.448	18068	4.2
H	R.	Print Preview		Н	125.0000		MS-MS	2.448	199967		125.0768	125.0768	100.1	3.8		2.448	14401	3.7
H	-			H	25,0000		MS-MS MS-MS	2.455	9246 48582		4.2831	4.2831	98.2	3.5	₩	2.453	17834	4.4
		Batch Properties		H	20.0000		MS-MS	2.100	10002		24.0077	24.0077	00.2	4.0		2.101		0.0
		1: C:\500_updated\DrugsOfAbuse_B0500\iiii_Test_01.batch.l	oin				MS-MS	2.460	9735		4.3735	4.3735		3.6		2.459	20051	3.6
		2: C:\op\QTOF\Verapamil-targetedMSMS\iiii_Test_01.batch.b	in	Щ			MS-MS	2.446	24841		10.9299	10.9299		3.9		2.445	20472	3.6
		3: C:\0500_updated\DrugsOfAbuse_B0500\iii_Test_01.batch	bin															
		4: C:\updated\DrugsOfAbuse_B0500\DrugsOfAbuseDemo.ba	tch.bin															
		Exit																

Figure 10. Export results

Task 3. Export and Print Results

Step 2 View the batch results as they appear in Excel; then exit Excel.

- 1 Note what is exported and what is not.
- 2 Close Excel when you are finished.

		🤈 - (≃ - =								iii_	Test_01.xlsx	- Microsoft	Excel					_	_
Fi	le	Home Ir	nsert Page Lay	out For	mulas	Data Revie	w View M	ASSHU	NTER REI	POR	TING Ad	d-Ins A	crobat						
ľ		Cut	Microsoft San	s Ser = 8	• A		≫~ ≣ w	rap Text		C	General	Ŧ					+		ĸ
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	Clip	oboard	G .	Font		rs,	Alignment		13		Numbe	r G		St	yles	-		Cel	ls
		A1	- (*)	Sample	2														
1	Α	B C	D	E	F	G	Н	1	J	K	L	М	N	0	Ρ	Q	R	S	Т
1			Sam	ple)	Amp Method				Amp Result	s		(136.2	2 -> 119.4	hp-d5 (IS	TD) Resu	141.1	-> 124
2		Name	Data File	Туре	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	RT	Resp.	Ratio	MI
3		Blank-1	CMAMBIk_01.d	Blank	i	*****		í		##					FALSE			1	###
4		Calib-L1	CMAMCal_L1.d	Cal	L1	******	2.5	2.14	657.86	##	2.481804	2.481804	99.27216	24.3	FALSE	2.128	1411.6	25.6	###
5		Calib-L2	CMAMCal_L2.d	Cal	L2	******	5	2.14	1073.4	##	4.92750484	4.9275048	98.5501	33	FALSE	2.128	1304.21	25.8	###
6		Calib-L3	CMAMCal_L3.d	Cal	L3	*****	12.5	2.134	2686.4	##	12.5996995	12.599699	100.7976	26.6	FALSE	2.122	1382.59	26.2	###
7		Calib-L4	CMAMCal_L4.d	Cal	L4	*****	25	2.02	5138.7	##	25.3646582	25.364658	101.4586	28	FALSE	1.99	1350	27.9	###
8		Calib-L5	CMAMCal_L5.d	Cal	L5	*****	125	2.102	19277	##	124.634283	124.63428	99.70743	27.1	FALSE	2.078	1053.49	26.4	###
9		QC-L2	CMAMQC_L2.d	QC	L2	****	5	2.14	1010.9	##	4.41037325	4.4103733	88.20747	27.6	FALSE	2.128	1352.25	31.2	###
10		QC-L4	CMAMQC_L4.d	QC	L4	****	25	2.134	4721.2	##	26.3121136	26.312114	105.2485	25.8	FALSE	2.122	1196.84	31.1	###
11		Sample-1	CMAMSam_01.d	Sample		****		2.027	38.431	##					FALSE				###
12		Sample-2	CMAMSam_02.d	Sample		****		2.14	1004.9	##	3.94583625	3.9458363		30.8	FALSE	2.128	1478.28	25.1	###
13		Sample-3	CMAMSam_03.d	Sample		******		2.102	2590.7	##	13.0294532	13.029453		25.3	FALSE	2.09	1291.64	29.6	###

Figure 11. Batch table in Excel

Step 3 Preview printouts for Batch Table and Compound Information data:

- 1 In Excel, click File > Print.
- 2 Inspect the **Print Preview** window to make sure it looks the way you want it.
- 3 Click File > Print.
- 4 Repeat steps **step 1-step 5** in **"Export the batch file iii_Test_01."** on page 50 for the compound information.
- 5 If you are not moving on to Exercise 4, click **File > Save Batch**.
- 6 Click File > Exit.

You can also print the **Batch Table** from the **Print Preview** program by clicking the **File > Print** menu item in the **Print Preview** program.

Task 3. Export and Print Results

Task 1. Adjust the Calibration Curve Fit 54

Task 2. Integrate Without Parameters 56

Task 3. Detect Outliers 70

In this exercise, you will use three tools to help you evaluate and obtain more accurate quantitation results:

- Curvefit Assistant, which calculates all combinations of curves and presents results with an equation and confidence band
- Parameterless integrator, so you don't have to figure out the parameters to change to improve the integration
- Outlier messages to help you easily detect result values that are out of the specified range

The DrugsOfAbuse batch is used in this exercise. The same tasks can be performed with Triple Quad data files, Q-TOF data files, and TOF data files.

Task 1. Adjust the Calibration Curve Fit

Task 1. Adjust the Calibration Curve Fit

This task shows you how to find the accuracy outlier for a compound, adjust its curve fit, and reanalyze the batch.

Step 1 If necessary, open the batch file *iii*_Test_01.batch.bin:

- 1 Click the **Quantitative Analysis (QQQ)** icon on your desktop to start the Quantitative Analysis program.
- 2 Click **Open Batch** on the toolbar to display the **Open Batch** dialog box.
- 3 Navigate to \Your Directory\DrugsOfAbuse and click iii_Test_01.batch.bin.

You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (QQQ)** from the Start menu.

If the default layout is not present, click **Restore Default Layout** on the toolbar before opening the batch.

Step 2 Find the accuracy outlier for amphetamine, and change the curve fit:

1 Make sure the **Batch Table** is set to single compound display mode, and the displayed target compound is **Amp**. See boxed portions of the illustration below.

Compound: Amp V ISTD: Amp-d5

- 2 Point to the cell in the **Calib-L1** row and the **Accuracy** column to display the Outlier message as shown below. Cells containing outliers can be in red (high) or blue (low).
- 3 In the **Calibration Curve** window, set **Origin** to **Ignore**, and **Weight** to **1/y**. The program displays a new curve fit formula and R² value.

Calibration Curve			_		_
🗄 🛃 \leftrightarrow 🌲 👯 📲 📾 🔛 Type: Linear	-	Origin: Ignore	-	Weight: 1/y	-
Amp - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 2 QCs 8 x10 1 y = 7.067567 * x + 0.129309 8 R ² = 0.99959614 1.6 Type:Linear, Origin:Ignore, Weight 1/y					

Curve Fit Origin

- Force Forces the curve fit line to go through the origin point (X=0, Y=0).
- Ignore Does not force the curve fit line to use the origin point (X=0, Y=0).

Task 1. Adjust the Calibration Curve Fit

Curve Fit Weight

- None Gives equal weight to all data points.
- **1/Y** Applies the formula 1/Y to the data points. This formula reduces the influence of high Y values while boosting the influence of low Y values.

Step 3 Analyze the batch and inspect the results in the Batch Table:

- 1 Click the **Analyze Batch** icon in the toolbar **Gamma** to analyze the batch.
- 2 Inspect the results in the **Batch Table** after batch analysis.



Step 4 Find accuracy outliers, if any, for other compounds:

- 1 Click **Next Compound** in the **Batch Table** toolbar in to view individual compounds, such as Cocaine, MDMA, and Met.
- 2 Examine the quantitation results, especially the values in the **Accuracy** column.

Note that the Accuracy value for the Calib-L3 standard for methamphetamine is out of the specified range.

Step 5 Change the curve fit for methamphetamine, and analyze the batch:

- 1 In the **Calibration Curve Fit** window, set **Origin** to **Ignore**, and **Weight** to **1/y**. The Quantitative Analysis program displays a revised curve fit formula and R² value.
- 2 Click Analyze Batch in the main toolbar to analyze the batch.

The Batch Table displays the new results after batch analysis.

Task 2. Integrate Without Parameters

Task 2. Integrate Without Parameters

This task shows you how to inspect data for proper integration. You learn how to perform the following tasks:

- Add integration columns to the Batch Table
- · View default integration values
- Closely examine the chromatogram, looking for such details as:
 - Outlier messages
 - Baseline parameters
 - Peak labels

Step 1 Add integration columns to the Batch Table:

- 1 Right-click anywhere in the **Batch Table**, and click **Add/Remove Columns**. The system displays the **Columns** dialog box.
- 2 From the Select Columns From drop-down list, select Compound Method.

Task 2. Integrate Without Parameters

3 From the **Available Columns** list, select **Int.** (Integrator Type) and **Int. Parms.** (Integrator Parameters) and click **Add**.

The Quantitative Analysis program moves the selected columns to the **Show** these columns in the order list.

This task assumes that the batch, *iii*_Test_01, is already open. If it is not, see step 1 in "Task 1. Adjust the Calibration Curve Fit" on page 54.

Columns				% X
Select Columns From:				
Compound Method	•		ċ	
Available Columns:			L	Show these columns in the order:
FWHM Limit High FWHM Limit Low	*	Add ->		Exp. Conc.
High m/z HitID		<- Remove		Int. Parms.
Ion Polarity Ion Source	-1	Add All ->>	h	
IPM ISTD Cmpd. ID	=	< Remove All		
ISTD Conc. ISTD Flag				
ISTD Resp. Limit High ISTD Resp. Limit Low				
ISTD Resp. Max. % Dev. Left RT Delta				
Library RT				
	Ŧ			
,				
				Move Up Move Down
		OK R	ese	t Default Cancel

- 4 From the Select Columns From drop-down list, select Compound Results.
- 5 From the Available Columns list, select Int. Metric (Integrator Metric) and click Add.

The system moves the selected column to the **Show these columns in the order** list.

Task 2. Integrate Without Parameters

6 Click OK.

Columns		? 💌
Compound Results	ſ	Show these columns in the order:
BL Std. Dev. Custom Calc. Est. Conc. FWHM Grp. # Height Int. End Int. Ketric Flag Int. Statt ISTD Conc. Ratio ISTD Resp. % Dev. ISTD Resp. Ratio Mass Accuracy Matrix Spike % Dev. Matrix Spike % Dev. Matr	Add -> <- Remove Add All ->> < Remove All	RT Resp. MI Calc. Conc. Final Conc. Accuracy Int. Metric
	OK <u>R</u> ese	Move Up Move Down

Step 2 View the default integration values for amphetamine:

- 1 Click **Previous Compound** in the **Batch Table** toolbar **to** view amphetamine (**Amp**).
- 2 Examine the default values in the Int. and Int. Parms columns in the Batch Table.

Note that the integrator used is the MS-MS integrator, which does not need you to enter parameters. That is why the **Int. Parms** column is blank.

Int.	Int. Parms.
MS-MS	

Task 2. Integrate Without Parameters

3 Examine the default values in the Int. Metric column in the Batch Table.

These values reflect the default integration quality metric used for the target compound Amp.

	Amp Method					Amp	Results		_
Exp. Conc.	Int.	Int. Parms.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric
	MS-MS								
2.5000	MS-MS		2.141	658		2.4161	2.4161	96.6	Accepted
5.0000	MS-MS		2.140	1059		4.8556	4.8556	97.1	Accepted
12.5000	MS-MS		2.134	2673		12.8162	12.8162	102.5	Accepted
25.0000	MS-MS		2.022	4952		25.9394	25.9394	103.8	Accepted
125.0000	MS-MS		2.101	18605		124.0262	124.0262	99.2	Accepted
5.0000	MS-MS		2.142	1006		4.3336	4.3336	86.7	Accepted
25.0000	MS-MS		2.135	4716		26.9911	26.9911	108.0	Accepted
	MS-MS								
	MS-MS		2.143	1004		4.0008	4.0008		Accepted
	MS-MS		2.105	2590		13.3556	13.3556		Accepted

Step 3 View integration problems for cocaine and MDMA:

Look for outlier messages at the intersection of the **Int. Metric** column and the **Blank-1** sample.

- 1 Close the Calibration Curve window:
- 2 Enlarge the chromatogram portion of Compound Information toolbar so that only the quantifier and qualifier chromatograms appear. Click the **Show/Hide Spectrum** icon.
- 3 Also click the Show/Hide ISTD icon.
- 4 Click the **Next Compound** icon in the **Batch Table** toolbar in the system displays the compound **Cocaine**.

Task 2. Integrate Without Parameters

5 Select the **Blank-1** row, and mouse over the word **Inspect** in the **Int. Metric** column for that row. The system displays any outlier message for that data, as well as the integrated chromatogram for cocaine.

	Agilen	t MassHunter	Quantitative Analy	/sis - DrugsOt	fAbuse	_80500 - iiii_Test_01												• 💌
: H	le Ed	it View Ana	lyze Method Up =	odate Repor	t loo	is Help	7											
			Analyze Batch	🕑 : Lay	out: 🛛		Kestore D	etau	It Layout									
Batc	h Table	e								_								- × >
	Sample	e: 👔 🛛 Blank-1	👻 🛃 🛛 Sar	mple Type: <	All>	Compound:	Cocaine			-	ISTD:	Cocaine-o	3			N	1 🖊 🎸	6
			Sar	mple				Coo	caine Method					Cocaine	Results			Qualifi
		V Name	Data File	Туре	Level	Acq. Date-Time	Exp. Conc.	IPM	Int.	Int. Parms.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric	Ratio
\mathbf{F}	0	🕅 🕅 🕐	CMAMBIk_01.d	Blank		5/12/2006 1:48 PM			MS-MS		2.433	20		11.8235	11.8235		Inspect	
		Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	2.5000		MS-MS		2.453	5189		2.3087	2.3087	92.3	Accepted	3.7
		Calib-L2	CMAMCal_L2.d	Cal	L2	5/12/2006 1:54 PM	5.0000		MS-MS		2.454	9716		4.2682	4.2682	85.4	Accepted	3.9
		Calib-L3	CMAMCal_L3.d	Cal	L3	5/12/2006 1:57 PM	12.5000		MS-MS		2.459	25187		11.5607	11.5607	92.5	Accepted	3.9
		Calib-L4	CMAMCal_L4.d	Cal	L4	5/12/2006 2:00 PM	25.0000		MS-MS		2.449	50649		25.2511	25.2511	101.0	Accepted	3.9
		Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM	125.0000		MS-MS		2.448	199967		125.0768	125.0768	100.1	Accepted	3.8
		QC-L2	CMAMQC_L2.d	QC	L2	5/12/2006 2:06 PM	5.0000		MS-MS		2.453	9246		4.2831	4.2831	85.7	Accepted	3.5
	-	QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	25.0000		MS-MS		2.455	48582		24.5377	24.5377	98.2	Accepted	4.0
	•	Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM			MS-MS		0.400	0705		4 0 7 0 7	4 0705			
		Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM			MS-MS		2.460	9/35		4.3/35	4.3/35		Accepted	3.6
		Sample-3	CMAMSam_03.d	Sample		5/12/2006 2:18 PM			MS-MS		2.446	24841		10.9299	10.9299		Accepted	3.9
-																_		
1							III											
Com	pound	Information																+ >
1	• ↔	\$ 🕂 🖂	i 👔 👢 🖛 🛛		ة الله													
+ MF	RM (30	4.1-> 182.0) C	MAMBIk 01.d			304.1->182.0_3	04.1 -> 82.0					+ MRM (2.41	1-2.480 min. 1	2 scans) (304	4.1->**) CI	AMBIk 01	d
2			2	.433 min.		- x10 ² Not For	und					[™] x10 1	1	82.0				
Ino	6-		1	I AA					Δ Δ	Δ		18						
0	4-					0.75	л	٨	- 11 11			0 4	1					
	2-	0		111664			A 11	η		'	AΛ	2						
	<u>_</u>	<u>(</u> `\			Ĺ		$\Lambda \Pi$	'n	- 1 V	U V (γY							304.1
	۰L	16 19	2 22	24 26	20	10	10 2		22 24	26 2	•	0		10 200	220 24	0 200	200	200
		1.0 1.0	2 2.2	Acquisition	1 Time	(min)	1.0 2		2.2 2.4 Ac	quisition Tin	ne (min)			200	220 24	. 200 M	ass-to-Chai	rge (m/z
+ ME	RM (30	7.1-> 185.0) C	MAMBIk 01.d			307 1 -> 185 0 3	071->850					+ MRM (2 37	5-2.481 min. 1	7 scans) (30	7.1->**) CI	AMBIk 01	d
2	2		2.4	403 min.		= x10 ² Not For	ind					≌ x10 1	1	85.0		. , .		1
15	21			Π		1.8						5	1					

- 6 Click the **Next Compound** icon in the Batch Table Standard toolbar or the Previous Compound icon in the Batch Table Standard toolbar until the system displays the compound MDMA.
- 7 Select the **Blank-1** row, and point to the **Int. Metric** column. The system displays any outlier message for that data, as well as the integrated chromatogram for MDMA.

The outlier message reads "MDMA: Integrator found the following problems with the peak at RT = 2.4664: Interference Problem."

Note that these colors appear for the integration metric:

Green - Accepted Blue - Inspect Red - Rejected

These colors are also reflected in the peak colors.

Task 2. Integrate Without Parameters

Step 4 Change the noise algorithm:

- 1 Right-click anywhere in the **Batch Table**, and click **Add/Remove Columns**. The system displays the **Columns** dialog box.
- 2 From the Select Columns From drop-down list, select Compound Method
- From the Available Columns list, select Noise Alg. (Noise Algorithm Type) and click Add.
 The system moves the selected column to the Show these columns in the order list.
- 4 Click OK.
- 5 Click the **Previous Compound** icon in the Batch Table toolbar in the system displays the compound **Amp**.
- 6 Examine the values in the **Noise Alg.** and **S/N** (signal-to-noise ratio) columns.

	Amp M	lethod					Amp	Results			Qualif	ier	Amp-d	5 (IST	Qualifier	
Exp. Conc.	Int.	Int. Parms.	Noise Alg.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric	Ratio	MI	RT	Resp.	Ratio	MI
	MS-MS		RMS													
2.5000	MS-MS		RMS	2.141	658		2.1151	2.1151	84.6	Accepted	24.3		2.129	1397	25.9	
5.0000	MS-MS		RMS	2.140	1059		4.5770	4.5770	91.5	Accepted	33.5		2.128	1298	25.9	
12.5000	MS-MS		RMS	2.134	2673		12.6107	12.6107	100.9	Accepted	26.7		2.121	1377	26.3	
25.0000	MS-MS		RMS	2.022	4952		25.8545	25.8545	103.4	Accepted	29.1		1.990	1304	28.8	
125.0000	MS-MS		RMS	2.101	18605		124.8426	124.8426	99.9	Accepted	27.0		2.076	1053	26.4	
5.0000	MS-MS		RMS	2.142	1006		4.0502	4.0502	81.0	Accepted	27.7		2.131	1356	31.1	
25.0000	MS-MS		RMS	2.135	4716		26.9159	26.9159	107.7	Accepted	25.6		2.121	1196	31.1	
	MS-MS		RMS													
	MS-MS		RMS	2.143	1004		3.7144	3.7144		Accepted	30.9		2.130	1445	25.7	

Step 5 Practice changing the noise algorithm from RSM to ASTM for amphetamine in the method. Exit, but don't save, the method:

1 Click **Method > Edit** to switch to method editing mode.

Task 2. Integrate Without Parameters

2 In the **Method Tasks** column, click **Advanced Tasks > Signal to Noise Setup**. The system displays the integrator parameters in the **Method Table**.

Method Tasks 🗸 🗸	¢
New / Open Method	1
Method Setup Tasks]
K MRM Compound Setup	
K Retention Time Setup	
11 ISTD Setup	
🪀 Concentration Setup	
T Qualifier Setup	
K Calibration Curve Setup	
🗳 Globals Setup	
Save / Exit	
🥁 Validate	
🔢 Save	
Save As	
K Exit	
Manual Setup Tasks	1
Outlier Setup Tasks]
Advanced Tasks	1
Integration Parameters Setup	
Signal to Noise Setup	
Smoothing Setup	
Mass Extraction Setup	
Isotopic Dilution Setup	
Compound Setup	
Compound 2D Setup	
Compound Library Setup	
B Rowse Acquisition Method	1

3 In the **Method Table**, click the drop down arrow in the **Noise Alg.** column for Amp.

A list of available noise algorithms appears.

4 Click ASTM.



- 5 Under Method Tasks/Save/Exit, click Exit.
- 6 At the **Would you like to apply this method to the batch?** prompt, click **No**. The system displays Batch Analysis mode.

Step 6 Turn off the baseline (highest concentration standard) and then back on for amphetamine. Compare the two chromatograms, one with the baseline on and the other with it off:

1 Select sample **Calib-L5** (if it is not already selected), and click the **Maximize Compound Information** icon in the toolbar.

Make sure that only the Compound Information pane is visible in the window.

Notice that the baseline is drawn in for the quantifier chromatogram as the default setting.

Ag	jilent N	/lassHunter	Quantitative Analy	sis - Drugs	OfAbuse	_B0500 - iii_Test_01				1
File	Edit	View Anal	yze Method Up	date Repo	rt Tool	s Help				
1 1			Analyze Batch	() Lay	out:		Restore D	efault Layou	it	
Batch T	able						-			
Sam	nple:	Calib-L	ō 🔻 🍋 San	nple Type: <	All>	- Compound:	🗺 Amp		-	ISTD:
Cor	mpoun	d Group: <	All> 👻	Sample (Group:	<all> -</all>	ISTD: <all></all>		Time Seg	ment: <all< td=""></all<>
			San	ple				Amp I	Nethod	
•	8	Name	Data File	Туре	Level	Acq. Date-Time	Exp. Conc.	Int.	Int. Parms.	Noise Alg.
0		Blank-1	CMAMBIk_01.d	Blank		5/12/2006 1:48 PM		MS-MS		RMS
		Sample-1	CMAMSam_01.d	Sample		5/12/2006 2:12 PM		MS-MS		RMS
and the second		Calib-L1	CMAMCal_L1.d	Cal	L1	5/12/2006 1:51 PM	2.5000	MS-MS		RMS
1000	*	Calib-L2	CMAMCal_L2.d	Cal	L2	5/12/2006 1:54 PM	5.0000	MS-MS		RMS
		Calib-L3	CMAMCal_L3.d	Cal	L3	5/12/2006 1:57 PM	12.5000	MS-MS		RMS
-	-	Colib 1.4	CMAMC5LL4.d	Cal	14	5/12/2000 2:00 PM	25.0000	MC MC	-	RMS
		Calib-L5	CMAMCal_L5.d	Cal	L5	5/12/2006 2:03 PM	125.0000	MS-MS		RMS
(and		QU-LZ	CMAMQC_L2.d	QC	LZ	5/12/2006 2:06 PM	5.0000	M2-M2		RMS
and the second s		QC-L4	CMAMQC_L4.d	QC	L4	5/12/2006 2:09 PM	25.0000	MS-MS		RMS
and the second s		Sample-2	CMAMSam_02.d	Sample		5/12/2006 2:15 PM		MS-MS		RMS
-		Sample-3	CMAMSam 03.d	Sample		5/12/2006 2:18 PM		MS-MS		RMS

2 Right-click either of the chromatograms to open the shortcut menu.

Task 2. Integrate Without Parameters

3 Click **Properties** at the bottom of the shortcut menu to open the **Properties** dialog box.

	ound information (2)				
General:			Retention time:		
Background color:	Automatic	•	Reference RT:	No display	
Foreground color:	Automatic	•	Recognition window:	No display	
Gridlines color:	No display	•	Peak purity:		
Time segment boundary:	No display	•	Show peak purity		
Chromatogram:			Purity colors		
Baselines					
Baseline calculation po	ints				
 Baseline calculation po Normalize quantifier 	ints				
Baseline calculation po Nomalize quantifier Original baselines after	ints manual integration				
Baseline calculation po Normalize quantifier Original baselines after Peak fill:	manual integration	•			
Baseline calculation po Normalize quantifier Original baselines after Peak fill: Fill colors	manual integration	¥			
Baseline calculation po Normalize quantifier Onginal baselines after Peak fill: Fill colors Peak labels	manual integration T5% Transparent	•			

- 4 Clear the Baselines check box in the **Properties** dialog box.
- **5** Click the **Apply** button and observe the peak without the baseline.

Notice that the baseline disappears after clearing the baseline check box.



6 Mark the **Baselines** check box in the **Properties** dialog box.

Task 2. Integrate Without Parameters

- 🚣 🚖 🔟 🔀 🖼 🗉 🕑 🖀 🛧 🗛 🔽 MRM (136.2 -> 91.4) CMAMCal_L5.d ≌ x10 3-l 2 101 min 3.4 3.2-3 2.8 2.6 2.4 2.2 2 1.8 1.6 1.4 1.2 1 0.8 0.6 0.4 0.2 0 -0.2 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6 2.8
- 7 Click the **Apply** button and observe the peak with the baseline drawn:

Step 7 Inspect the calculation points for the baseline for amphetamine:

- 1 Mark the Baseline Calculation Points check box in the Properties dialog box.
- 2 Click **Apply** and observe where the baseline starts and stops.



- 3 Clear the Baseline Calculation Points check box in the Properties dialog box.
- 4 Click **Apply** and observe the chromatograms.

Task 2. Integrate Without Parameters

5 Compare the chromatograms with and without Baseline Calculation Points.



Step 8 Display the peak labels for amphetamine.

- 1 From the **Properties** dialog box, click **Peak Labels**. The system displays the **Peak Label** dialog box.
- 2 Mark all the **Peak Labels** check boxes and the **Display Label Names** check box.
- 3 Click OK.

Peak Labels	×
Image: Conc. Image: Conc. Image: Final	Move Up Move Down
☑ Display Label Names (ex. RT=2.	452)
Display Units for Conc., RT and	Delta RT
ОК	Cancel

Task 2. Integrate Without Parameters

4 Click the **Apply** button in the **Properties** dialog box. The peak labels should now match those shown in the example below.



- 5 Click **Peak Labels** in the **Properties** dialog box. The system displays the **Peak Labels** dialog box.
- 6 Clear all the **Peak Labels** check boxes except **RT** (retention time). Clear the **Display Label Names** check box, and click **OK**.
- 7 Click **Apply** in the **Properties** dialog box and observe the change in Peak Labels.

Step 9 Display the qualifier chromatogram on the right-side before and after normalization:

1 Click the **Compound Information (2)** tab. In the **Qualifiers** area, mark the **Normalize** check box.

Task 2. Integrate Without Parameters

2 Click **Apply** and observe that the two peaks now converge and appear as one peak.

For B.04.01 and later revision: Notice that the default setting displays the normalized qualifier peak overlaid on the quantifier peak.



- 3 Clear the Normalize Qualifiers check box of the Properties dialog box.
- 4 Click **Apply** to display the qualifier second quantifier peaks again.



Task 2. Integrate Without Parameters

Step 10 View the uncertainty band:

- Select the type of uncertainty band you would like to display from the drop-down menu in the Uncertainty Band field of the Properties dialog box. Click Apply and the uncertainty band appears in the qualifier chromatogram.
- 2 Select **No display** from the **Uncertainty Band** drop-down menu of the **Properties** dialog box. Click **Apply** to remove the uncertainty band from the qualifier chromatogram.
- 3 Click **OK** to close the **Properties** dialog box.
- 4 Compare the qualifier chromatogram with and without the **Uncertainty band**. The Uncertainty band is a dashed band that shows the upper and lower boundaries for the qualifier abundance



Step 11 Remove the Int. and Int. Parms. columns from the Batch Table:

- 1 Click the **Restore Default Layout** button.
- 2 Right-click the **Compound Method** section of the **Batch Table**, and click **Add/Remove Columns**.
- 3 From the right-hand list, select Int. and Int. Parms. (Compound Methods).
- 4 Click **Remove**, and then **OK**.

Task 3. Detect Outliers

Task 3. Detect Outliers

This task shows you how to fine-tune the accuracy range for a compound and hide and show results with outlier flags.

Step 1 View outlier information for MDMA:

- 1 Click **Next Compound** in the **Batch Table** toolbar until the system displays the compound MDMA.
- 2 Select the **Blank-1** row, and point the cursor to the **RT** column, as shown in the example below.



Task 3. Detect Outliers

3 Examine the outlier information in the **Qualifier** ... **Results > Ratio** column for Sample 1, as shown in the example below.



Step 2 Change the accuracy range for amphetamine in the method, and reanalyze the batch:

- 1 Click the **Previous Compound** icon in the toolbar **[** until the system displays the compound **Amp**.
- 2 Select the Calib-L5 row in the table.
- 3 Click Method > Edit to switch to method editing mode.
- 4 In the Method Tasks column, click Outlier Setup Tasks > Accuracy.

Task 3. Detect Outliers

5 Set the Accuracy Max % Dev value to 5% for Amp.

You can split the **Method Table** by dragging the small rectangle to the left of the scroll bar. In the example below, the rectangle next to the bottom scroll bar was used to split the **Method Table**. The information in the two sections is exactly the same. You can use these two panes to look at two sections of the table at the same time.

He Agilent MassHunter Quantitative Analy	ysis	is - [New Method]					
Eile Edit View Analyze Method Upd	date	te <u>R</u> eport <u>T</u> ools <u>H</u> elp					
🗄 🛅 🕞 📕 🖬 🖓 🗛 Analyze Batch 🕴 🕢	; i	Layout: 🔜 🔛 🔛 🛄 🔼 🗭	Rest	ore <u>D</u> efault Layout			
Method Tasks	×	Method Table					;
Method Setup Tasks	^	Time Segment: 🖛 <all></all>		🝷 🔿 📔 Compound: 🛽	🛋 Amp-d5 🛛 💌 🛤	Reset Table View	
K MRM Compound Setup		Sample		1	1		
Retention Time Setup		Name		Acq. Date-Time	Data File		
ist ISTD Setup		Calib-L5		5/12/2006 2:03 PM	CMAMCal_L5.d]	
Concentration Setup		Quantifier					
T Qualifier Setup		Name	on	Scan	Туре	Accuracy Max. % Dev.	LOQ Accuracy Multiplier
Calibration Curve Setup		Amp	4	MRM	Target	5.0	1.
		► Amp-d5	4	MRM	ISTD	2U.U	1.
I Giobals Setup		Cocaine	2.0	MRM	Target	20.0	1.
Save / Exit		Cocaine-d3	5.0	MRM	ISTD	20.0	1.
🕅 Validate		MDMA dE	8.2	MRM	larget	20.0	1.
	-11	Meth	93	MRM	Tarnet	20.0	1.
Save		Meth-d5	3	MRM	ISTD	20.0	1
Save As			<u> </u>	1			
🔀 Exit							
Manual Setup Tasks							
Outlier Setup Tasks							
Accuracy							
🛣 Qualifier Ratio							
🖟 Retention Time							
Relative Retention Time	~	<	<				
	-1					4 Compounds	(4 total) 4 ISTD (4 total)

6 In the **Method Tasks** column, click **Save/Exit > Exit**, then select **None** under Additional batch processing after applying the method, and click **Yes** to the **Would you like to apply this method to the batch?** prompt.
5 Exercise 4: Use Three Tools to Evaluate Results

Task 3. Detect Outliers

7 Press F5 to analyze the batch. Red (high) and blue (low) outlier values now appear in the **Accuracy** column for Amp

You can also split the **Batch Table** into two sections. By default, the **Sample** columns are locked in position and only the other columns are scrolled. If you split the table into two sections, you can determine which columns appear in each section. You need to clear the **Lock Sample Columns** menu item in the Batch Table shortcut menu if you split the **Batch Table**.



Step 3 Using the following set of outlier flag icons : R R r r r r :

- 1 Click the **Display rows that have High outliers** icon on the toolbar to display only samples with high outliers.
- 2 Click the Turn off outlier filter 📷 icon to display all samples.
- 3 Click the **Display rows that have High/Low outliers** ricon on the toolbar to display only samples with low outliers.
- 4 Click the **Display rows that have High/Low outliers** ricon again to display all the samples.
- 5 Click the Select Outliers 🙀 icon to bring up the Outliers dialog box.
- 6 Clear the Accuracy and Retention Time check boxes, and click OK.
- 7 Click the Select Outliers 🙀 icon to bring up the Outliers dialog box.
- 8 Mark the Accuracy and Retention Time check boxes, and click OK.
 - Note that to restore the **Batch Table** to view all data files, with and without outliers, simply click again on the icon you selected for filtering outliers.

5 Exercise 4: Use Three Tools to Evaluate Results Task 3. Detect Outliers

Exercise 5: Generate Quantitation Reports

This exercise helps you learn how to do these tasks:

- · Generate report methods using one or more report templates
- Generate a report

The **DrugsOfAbuse** batch is used in this exercise. The same tasks can be performed with Triple Quad data files, Q-TOF data files, and TOF data files.

Each exercise is presented in a table with three columns:

- Steps Use these general instructions to proceed on your own to explore the program.
- Detailed Instructions Use these if you need help or prefer to use a step-by-step learning process.
- Comments Read these to learn tips and additional information about each step in the exercise.

The report method you develop determines the report you create in MassHunter. Report methods are made of one or more report templates combined and edited to meet your reporting requirements. When developing a report method, you can use either Excel or PDF templates. PDF templates are included with this release and can generate reports 20 times faster than Excel templates. In addition, they have more options for scalability and performance.

In this exercise, you will first develop a report method using PDF templates, and create single sample and batch reports using this same method.

Step 1 Open the batch file *iii_Test_01.batch.bin*:

- 1 To start the Quantitative Analysis program, click the **Quantitative Analysis** (QQQ) icon on your desktop.
- 2 Click **Open Batch** on the toolbar to display the **Open Batch** dialog box.

3 Navigate to **\Your Directory\DrugsOfAbuse** and click *iii_***Test_01.batch.bin**.

If the batch is already open, skip to step 2.

You can also access the program by clicking **Programs > Agilent > MassHunter Workstation > Quantitative Analysis (QQQ)** from the Start menu.

If the default layout is not present, click **Restore Default Layout** on the toolbar before opening the batch.

Step 2 Quantitate the samples for this batch and save your results:

- 1 With the batch table open, click the **Analyze Batch** button on the tool bar to generate results.
- 2 Click File > Save to save the batch.

Quantitative reports contain sample information generated during the batch. The reporting function will not work until sample results have been quantitated and saved.

If the batch is already quantitated, skip to "Create a PDF report method:".

Step 3 Create a PDF report method:

1 Click **Report > Generate** from the toolbar.

The system displays the **Generate Report** dialog box.

- 2 Accept the default **Report Folder** directory for this report.
- **3** Under the **Report Method** field, click the **New** button to create a new report method.
- 4 Click the **Add Template** button in the **Report Method Edit** dialog box to open the browser.
- 5 Navigate to the MassHunter/Report Templates/Quant/PDF-Reporting directory, select DrugAnalysis.report.xml and click Open.
 The program adds the template to the Template field in the Report Method Edit pane.
- 6 Repeat step 4 and step 5 to add DrugAnalysis_DopingScreening.report.xml.
 - You may change the destination directory for saving the report in the **Report Folder** field;

- The Report Method Edit feature of the software allows you to combine existing templates into a report method for developing an Excel or PDF report, or both.
- The software defaults to the last report method used for the last report generated. Rather than generate a new report method, you can use the default method if appropriate, or select a different existing method.
- To select an existing report method, click the **Choose** button under the **Report Method** field, and navigate to the folder to select your method.

Step 4 Edit the report method to create single sample and batch PDF reports:

- 1 In the **Report Method Edit** dialog box, on the **DrugAnalysis.report** template line, **Report Mode** field, select **Single Sample** from the drop down menu.
- 2 On the **DrugAnalysis _Doping Screening.report** template line, select **Batch** from the drop down menu in the **Report Mode** field.
- 3 Select your language from the drop down menu in the Language field.
- 4 Select a paper size from the drop down menu in the **Paper Size** field.
 - The **Report Method Edit** dialog box allows you to edit certain features of the templates you choose to include in the report method.
 - The PDF reporting option allows you to create English, Chinese, or Japanese reports. Excel reports are provided in English only so this option will be grayed out.
 - In Excel reports, there are limits on your paper size. PDF reports provides a choice.
 - You can also select your **Publish Format**. In PDF reports, there is only one Publish Format; therefore, this field is grayed out for this example.

Step 5 Select the way the system handles your report results:

- 1 Select the **Results** tab of the **Report Method Edit** window.
- 2 Under the Generate Reports results file field, click Auto.

3 From the drop down menu of the Instrument field, select QQQ.

It is recommended to use **Auto** in most cases. This limits the generation of an Excel file with the report to only those cases in which an Excel report is selected. PDF reports are quick and efficient when the generation of an Excel file is not necessary.

Step 6 Set the graphic setting options for the method:

- 1 Click the **Graphic Settings** tab to review the graphic settings.
- 2 Select the Generate graphic file check box to add graphics to your report.
- **3** Leave the default settings for the rest of the graphic setting fields.

The **Graphic Settings** tab allows you to specify the appearance of the graphics in your report by editing the **Quantifier/Qualifier Overlay chromatogram, Spectra, Sample chromatogram, Calibration Curves** and **Fixed range graphic settings**. If you do not change the settings, the software will provide default settings appropriate for your data.

Step 7 Save the report method:

- 1 Click the save icon in the **Report Method Edit** window.
- 2 Name the report method **DOA.m**.

You must save the method before you can close the window and generate a report.

Step 8 Close the Report Method Edit window:

1 Click Save & Exit to close the Report Method Edit dialog box to return to the Generate Report window.

Generate Report		×
Batch file:		
Batch folder:	C:\MassHunter\Data\DrugsOfAbuse\	
Batch file:	DOA_Demo.batch.bin	Browse
Report folder:		
C:\MassHunter\Data\Drugs0	0fAbuse\QuantReports\DOA_Demo-60	Browse
Report method:		45
C:\MassHunter\Report Temp	plates\Quant\PDF-Reporting\DOA.m	
	Choose New	Edit
Samples/Compounds:	· · ·	
💟 All samples	Choose samples	
All compounds	Choose compounds	
Generate:		
Generate reports now		
💮 Queue report task		
Start Queue View	er	
	ОК	Cancel

Step 9 Generate a report from the method:

- 1 Verify that the method you just created is in the **Report Method** field.
- 2 In the **Samples/Compounds** field, uncheck **All Samples**, to open the batch table.
- 3 Highlight one of the samples in the batch table window and click OK.
- 4 Click **All Compounds** to show all the compounds in the sample you have selected.
- 5 Select Generate reports now and click OK to generate the report.
 - You can choose to show all the samples and all the compounds in the batch, or select specific samples or compounds in the batch table to show in your report.

- PDF reports generate quickly so Generate the report now is the best option to obtain the report right away. If you are generating an Excel file along with the report, you can select Queue report task to view the progress of the report it is generating.
- All reports generated are displayed in the viewer. The most recent display at the top of the list.
- Reports are viewed or printed from the Excel or the PDF file you have created.

Name	Date modified	Туре	Size
DrugAnalysis_000_CMAMBlk_01.pdf	2/5/2013 10.96 AM	PDF Complete Do	95 KB
Signal Drug Analysis_001_CMAMCal_L1.pdf	2/5/2013 10:56 AM	PDF Complete Do	99 KB
DrugAnalysis_002_CMAMCal_L2.pdf	2/5/2013 10:56 AM	PDF Complete Do	99 KB
NrugAnalysis_003_CMAMCal_L3.pdf	2/5/2013 10:56 AM	PDF Complete Do	100 KB
NrugAnalysis_004_CMAMCal_L4.pdf	2/5/2013 10:56 AM	PDF Complete Do	100 KB
NrugAnalysis_005_CMAMCal_L5.pdf	2/5/2013 10:56 AM	PDF Complete Do	100 KB
S DrugAnalysis_006_CMAMQC_L2.pdf	2/5/2013 10:56 AM	PDF Complete Do	99 KB
DrugAnalysis_007_CMAMQC_L4.pdf	2/5/2013 10:56 AM	PDF Complete Do	99 KB
NugAnalysis_008_CMAMSam_01.pdf	2/5/2013 10:56 AM	PDF Complete Do	98 KB
NrugAnalysis_009_CMAMSam_02.pdf	2/5/2013 10:56 AM	PDF Complete Do	99 KB
💈 DrugAnalysis_010_CMAMSam_03.pdf	2/5/2013 10:56 AM	PDF Complete Do	99 KB
💈 DrugAnalysis_DopingScreening.pdf	2/5/2013 10:56 AM	PDF Complete Do	166 KB

Step 10 View the report:

1 Double-click on a file to open and display the report.

Alternatively, you may open the report by selecting the file in Windows Explorer.

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Ten Main Capabilities

Ten Main Capabilities

Quantitative Analysis includes ten capabilities that help you integrate, quantitate, and review your data more easily and powerfully:

Batch-at-a-Glance: Batch Table Setup

- New batch Creates a batch table in which you can operate on samples and compounds from a single view
- Analyze Recreates the calibration curve and requantitates all samples using the method that is currently open
- Quantitate Applies the existing calibration curve to the current batch, sample, or compound

The granularity of applying quantitation allows you to quickly manipulate a particular signal.

• Integrate – Integrates signals to the current batch, sample, or compound

Method Editor

- MRM Setup Presents a quantitation method in simple stepwise fashion
- Create method from acquired MRM data Creates a quantitation method automatically from the acquisition method after requiring only the assignment of ISTD relationship and concentrations
- Create a method manually using the graphics in the Sample Information window
- Group by time segment Organizes methods by compounds in ordered time segments
- Validate Ensures that a quantitation method meets rigorous criteria
- Isotopic dilution Supports adjustments from (Rx, Ry) Colby constant calculations

Calibration

- CurveFit assistant Calculates all combinations of curves; picks disabled points; and presents results with an equation that is sortable by confidence band and custom filterable by R², standard error, and max % residual
- Dilution assistant Calculates and creates calibration levels based on a default or specified serial dilution scheme

Ten Main Capabilities

- Copy Cal levels Copies calibration levels from one compound to other compounds
- Disable Cal points Disables calibration points based on level, or individual compounds in tables, or interactively through graphs
- Curve fits Supports curves by:
- Type: Linear, Quadratic, First order In, Second order In, Average of Response Factors
- Origin: Ignore, Include, Force, Blank Offset
- Weight: None, 1/x, 1/x², 1/y, 1/y², Log, 1/SD²
- Replace curve Creates calibration curves from existing calibration samples
- Average replicates Averages replicate levels in the method calibration table.
- Import levels Imports calibration levels and concentrations from a file
- Scale graphs Provides graphs with the capability to be autoscalable by X, Y, X-log, and Y-log; and intelligent zooming to fit specified levels

Integrator

- Agile and Agile2 integrator Provides a parameter-free integrator at all levels of signals that reduces manual integration efforts
- Integrator metrics Generates metrics that characterize the signal's integration to accept, inspect, or reject the integration
- Signal-to-noise Calculates signal-to-noise for peaks
- Graphics Shows superior interaction with the graphing of a compound and the display of peak information

Batch-at-a-Glance: Results

- Navigation Moves (previous, next, direct) between samples, compounds, time segments, and compound groups
- Compound views Switches between the details of the current compound/sample or the summaries of multiple compounds/samples
- Batch Table views Enables flat-table layouts or the capability to drill down to vertically or horizontally nested tables for details and compound table layout
- Window layout Reorganizes the screen to its defaults, or saves or loads custom-window layouts

Ten Main Capabilities

- AutoReview Displays each sample automatically and interactively, allowing you to stop at any time for closer inspection
- Columns Enables you to add, remove, reorder, save, load, restore, or reset columns
- Float pane Floats any pane onto another monitor to enable dual-monitor presentations
- Export Table Exports Batch-at-a-Glance tables directly to Excel files
- Export Graphics Exports any graphic to a customized size in multiple formats
- Copy/Paste Copies or pastes any graphic directly into Microsoft Office applications such as Word, PowerPoint, Excel, etc.
- Print/Preview Prints or previews screen content in WYSIWYG format (what-you-see-is-what-you-get)
- Filter Displays any combination of sample types
- Sort Sorts any column that appears in a table

Compounds-at-a-Glance: Results

- Print/Preview Prints or previews compound chromatograms.
- Copy/Copy Page Copies selected compound chromatograms, or all compound chromatograms on the screen into Microsoft Office applications such as Word, PowerPoint, Excel, etc.
- Edit Compound Chromatograms Manually integrate the data, or select zero-peak compounds.
- Views Displays chromatogram details such as baselines, filled peaks.
- Adjust Axes Link/Unlink X or Y axes, autoscale to fit the panes, fit to peaks or fit to calibration levels.
- Layout Organize rows by compounds or samples, select chromatogram overlays, review sample by sample or compound by compound, set display options.
- Highlight Compounds with outliers

Outlier Detection

- Manage Sets up and selects specific outliers that can be detected and individually controlled
- Highlight Highlights outlier values (high = red, low = blue) in the results table

Ten Main Capabilities

- Filters Lets you display the results of selected types of filters
- Outliers Supports specific types of data for outlier detection
- Quantitation message Warns you of samples that encountered serious problems during quantitation

Report

- Generate Generates graphics and report results for importing and formatting for Excel XML
- Custom Lets you customize the Excel template
- PDF Reporting Lets you customize and generate PDF Reports

Update

- Update/Average RT Updates or calculates weighted averages of the compound's retention times
- Update Qualifier Ratios Updates qualifier ratios based on the compound's current sample
- Update Mass Assignments Updates mass assignments based on compounds current sample

Qualitative

- Sample Information lets you display the chromatogram and extracted spectra for the current sample
- Chromatogram/Spectrum Provides significant features that can be used to explore spectra for different types of signals

Quantitative Methods

Quantitative Methods

The Method Editor lets you create a new quantitation method from an MRM acquisition data file (**Figure 12**), from SIM data, from an acquired Scan data file, or manually.



Figure 12. Quantitative view – Method Editor

A file selected from the Batch Table is used as a reference for developing the method settings. These settings are then used to generate the calibration curve and quantitate the standards, QCs, and samples.

Parameter-Free Integrator

Parameter-Free Integrator

What is the parameter-free integrator?

Agilent has developed a new peak integrator algorithm that works especially well for MS/MS data. The parameter-free integrator presents these advantages:

- Handles low-level noisy data by setting a peak's starting and ending points statistically
- Adjusts the threshold automatically
- Eliminates the need for manually reintegrating peaks for low-level MRM signals
- Identifies those peaks that appear reliable and those that should be discarded

Example of integration results

Figure 13 shows data at two extremes.



Figure 13. Parameter-free integrator – Data at two extremes

Parameter-Free Integrator

The lower chromatographic peak could be easily integrated since it is a nice Gaussian-shaped peak, but it would be difficult to define the baseline of the upper peak. In fact, many integrator algorithms might interpret these results as multiple peaks.

However, Agilent's new algorithm had no trouble defining the baseline and recognized this as a single peak. In fact, the new integrator algorithm would integrate this as a single peak even if the baseline were rising, instead of being flat, as shown.

Batch-at-a-Glance: Results

Batch-at-a-Glance: Results

The integration results obtained from the analysis of amphetamine (Amp) are shown in **Figure 14**. This is a flat view of the **Batch Table**, **Compound Information**, and **Calibration Curve**.





- The **Batch Table** shows the integration results from applying the quantitation method to each data file. Colored highlights correspond to results that are lower (blue) or higher (red) than expected.
- The **Compound Information** window at the lower left displays the integrated chromatographic peaks.
- The **Calibration Curve** is shown at the lower right.

Compounds-at-a-Glance

Compounds-at-a-Glance

The Compounds-at-a-Glance view shows specific compounds detected in each sample, as shown in **Figure 15**. This feature allows you to view the compound chromatograms and arrange them for easy data analysis. It is especially useful for food safety labs that look for compound trends within batches of samples.



Figure 15. Compounds-at-a-Glance in Quantitative Analysis

The setup feature in the Compounds-at-a-Glance allows you to select the compounds and samples you would like included in the view. As shown in **Figure 16** the different tabs at the top of the **Setup Graphics** box provide different options for selecting and arranging the chromatograms.

- The **Samples** tab lists all the samples included in the batch, and gives options for selecting all samples or specific samples.
- The Compounds tab lists the compounds detected in the batch. It allows you to choose the compounds you would like to view.

Compounds-at-a-Glance

- The **Organize** tab allows you to specify the arrangement of the chromatograms, according to sample and compound. It provides overlay options for compounds, samples, and outliers. The tab gives choices for adjusting the chromatograms, such as displaying baselines or fill peaks to best illustrate compound detection trends.
- The **Outlier** tab provides options for showing outliers in the data.

Name	Data File	Туре	Level	Sample Group	Matrix Spike Group
Sample-1	CMAMSam_01.d	Sample			
•		III			
	Add >	Add All >>	< Remove	<< Remove All]
Samples shown	n in this order:				_
Samples shown	n in this order: Data File	Туре	Level	Sample Group	Matrix Spike Group
Samples shown Name Calib-L3	n in this order: Data File CMAMCal_L3.d	Type Cal	Level	Sample Group	Matrix Spike Group
Samples shown Name Calib-L3 Calib-L4	Data File CMAMCal_L3.d CMAMCal_L4.d	Type Cal Cal	Level L3 L4	Sample Group	Matrix Spike Group
Samples shown Name Calib-L3 Calib-L4 QC-L2	Data File CMAMCal_L3.d CMAMCal_L4.d CMAMQC_L2.d	Type Cal Cal QC	Level L3 L4 L2	Sample Group	Matrix Spike Group
Samples shown Name Calib-L3 Calib-L4 QC-L2 QC-L4	h in this order: Data File CMAMCal_L3.d CMAMCal_L4.d CMAMQC_L2.d CMAMQC_L4.d	Type Cal Cal QC QC	Level L3 L4 L2 L4	Sample Group	Matrix Spike Group
Samples shown Name Calib-L3 Calib-L4 QC-L2 QC-L4 Sample-2	h in this order: Data File CMAMCal_L3.d CMAMCal_L4.d CMAMQC_L2.d CMAMQC_L4.d CMAMQC_L4.d	Type Cal Cal QC QC Sample	Level L3 L4 L2 L4	Sample Group	Matrix Spike Group
Samples shown Name Calib-L3 Calib-L4 QC-L2 QC-L4 Sample-2 Sample-3	h in this order: Data File CMAMCal_L3.d CMAMCal_L4.d CMAMQC_L2.d CMAMQC_L4.d CMAMQC_L4.d CMAMSam_02.d CMAMSam_03.d	Type Cal Cal QC QC Sample Sample	Level L3 L4 L2 L4 L4	Sample Group	Matrix Spike Group
Samples shown Name Calib-L3 Calib-L4 QC-L2 QC-L4 Sample-2 Sample-3 Calib-L5	h in this order: Data File CMAMCal_L3.d CMAMCal_L4.d CMAMQC_L2.d CMAMQC_L4.d CMAMQC_L4.d CMAMSam_02.d CMAMSam_03.d CMAMCal_L5.d	Type Cal Cal QC QC Sample Sample Cal	Level L3 L4 L2 L4 L4 L4 L5	Sample Group	Matrix Spike Group

Figure 16. Setup options for Compounds-at-a-Glance

Compound Confirmation

Compound Confirmation

The format shown in Figure 17 can be of value to certified drug-testing laboratories. It shows two sets of plots that can be obtained from a THC analysis.



Amphetamine qualifier ion - Normalized

Amphetamine qualifier ion - Not normalized



Two product ions must be acquired for confirmation: a quantifier ion and a qualifier ion. Typically, the quantifier ion that is used for quantitation is the most abundant of the two product ions.

To be able to confirm the presence of Amphetamine, the qualifier ion peak area must be at least a certain percentage of the quantifier ion, a number that is set in the quantitation method. In this example, 26.5% is used with a window of \pm 20%. This means that the area of the qualifier ion must be in the range of 21.2 to 31.8% of the quantifier ion for the analyte Amp. The qualifier for the ISTD, or Amp-d5, also has a specific range that it must be in.

From the figure on the left, whether or not the qualifier ion falls within the accepted window is not easily determined because the size of the qualifier peak is normalized by a factor of 1/0.265. In the figure on the right, the acceptance window is centered at 26.5% of the quantifier ion peak and the qualifier ion is drawn not normalized, or on the same scale as the quantifier. If the ion is not

Compound Confirmation

within the required acceptance window, then it is shaded blue, but is still transparent so as not to hide the quantifier ion. This makes it easier to confirm the presence of compounds visually.

Compound Calibration

Compound Calibration

The Quantitative Analysis program contains several tools to help calibrate and quantitate compounds:

- CurveFit Assistant
- Cursor Pointer for Data Point Information
- Data Point Zooming

CurveFit Assistant

The CurveFit Assistant provides an analytical view of evaluating the possible curve fits (Figure 18).



Figure 18. CurveFit Assistant

Compound Calibration

Note that the black line drawn through the data points uses Quadratic as the Fit, 1/x as the Weight, and Include as the Origin as shown at the top. Many other combinations of the curve settings are listed below the calibration curve, with the selected one highlighted in blue. The highlighted settings are also plotted in blue in the curve window.

You can find the best curve fit, for example, one that corresponds to the highest R^2 value, by ordering all of the possible results from the best to the worse R^2 values and then deciding how many data points to consider as being outliers.

For example, the first set of parameters in the list corresponds to a Linear Fit, Ignore Origin, and Equal Weight. The corresponding R^2 value is 0.9998001477, which is very good. The corresponding curve can be plotted by simply clicking this entry in the table.

Using these settings, data can be requantitated. Eliminating outliers is common as a standard operating procedure (SOP) in some laboratories.

Data point information

Overlapping data points are not unusual in a calibration curve, especially with triple quad MS data, where %RSD values are quite low (**Figure 19**). To help distinguish the data points from one another, the cursor can be moved over the data points to obtain more information about them.



Figure 19. Amp results: Calibration data point information

Compound Calibration

This figure shows two examples of this type of information. The first example shows that the data points overlap and advised you to zoom in to see them separately. The second example shows information on the data point itself.

Data point zooming

You can zoom in on overlapping data points to see individual data points not visible in the visual presentation.

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