

Agilent 6410 Triple Quad LC/MS

Familiarization Guide



Notices

© Agilent Technologies, Inc. 2006-2007

No part of this manual may be reproduced in any form or by any means (including electronic storage and retrieval or translation into a foreign language) without prior agreement and written consent from Agilent Technologies, Inc. as governed by United States and international copyright laws.

Manual Part Number

G3335-90021

Edition

Third Edition, August 2007

Printed in USA

Agilent Technologies, Inc. 5301 Stevens Creek Blvd. Santa Clara, CA USA 95051

Software Revision

This guide is valid for the B.01.03 or later revision of the Agilent MassHunter software for the Agilent 6410 Triple Quad LC/MS, until superseded.

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.720-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In this Guide...

The Familiarization Guide presents step-by-step exercises to help you learn to use the Agilent Triple Quad LC/MS. You can do these exercises with the demo data files, SulfaDrugs (Exercise 1) and DrugsOfAbuse (Exercises 2 through 5), shipped with the system (in the **Data** folder of your software CD), or with data you acquire.

1 Develop an acquisition method for the Agilent Triple Quad LC/MS

In this exercise, you learn how to determine optimum Agilent Triple Quad MS acquisition settings for analyzing your compounds of interest, including the fragmentor voltage and collision energy.

2 Set up and quantitate a batch of acquired datafiles

In this exercise, you set up a batch table, a quantitation method, and target compounds, using acquired datafiles. Finally, you analyze the batch and save the results.

3 Review quantitation results

In this exercise, you inspect the sample and compound data in a batch file, customize layouts and export your batch results to a Microsoft Excel file.

4 Use three new tools to evaluate results

The new tools in this exercise make it easier for you to evaluate and obtain more accurate quantitation results.

5 Work with quantitation reports

In this exercise, you generate reports using specified templates, review reports in Microsoft Excel and customize the report template.

Before you start...

This guide assumes that the Agilent MassHunter Workstation Acquisition and Quantitative Analysis applications have been installed, and the LC modules and the 6410 Triple Quad LC/MS have been configured. Also, the performance has been verified, and the system has been turned on. If these actions have not yet been done, see the *Agilent 6410 Triple Quad LC/MS System Installation Guide*.

The exercises in this guide use this equipment and materials:

- Agilent 1100 LC modules: well-plate sampler, binary pump, thermostatted column compartment, DAD
- Zorbax, SB-C18 2.1mm x 30mm, 3.5um, 100Å, p/n 873700-902.
- A 10-µL sulfa mix sample, prepared as directed in Exercise 1.

Contents

Contents

Exercise 1 Develop an acquisition method for the Agilent Triple Quad LC/MS 7

Before you begin... 9

- Task 1. Enter acquisition parameters and acquire data 11
- Task 2. Determine precursor ion masses 15
- Task 3. Find optimum fragmentor voltage for maximum response
 18
- Task 4. Determine product ion masses 28
- Task 5. Find optimum collision energy for MRM acquisition 35

Exercise 2 Set up and quantitate a batch of acquired data files 39

- Task 1. Set up a new batch 41
- Task 2. Set up a new method for the batch 44
- Task 3. Set up target compounds 47
- Task 4. Set up quantitation 50
- Task 5. Analyze and save the batch 56

Exercise 3 Review quantitation results 57

- Task 1. Navigate the Batch Table results 58
- Task 2. Change result window layouts 63
- Task 3. Export and print results 70

Exercise 4 Use three new tools to evaluate results 73

- Task 1. Adjust the calibration curve fit 74
- Task 2. Integrate without parameters 77
- Task 3. Detect outliers 89

Exercise 5 Work with quantitation reports 93

- Task 1. Generate quantitation reports 94
- Task 2. Review the reports 99
- Task 3. Customize a report template 101

Contents



Agilent 6410 Triple Quad LC/MS Familiarization Guide

Exercise 1 Develop an acquisition method for the Agilent Triple Quad LC/MS

Before you begin... 9

Task 1. Enter acquisition parameters and acquire data 11
Task 2. Determine precursor ion masses 15
Task 3. Find optimum fragmentor voltage for maximum response 18
Task 4. Determine product ion masses 28
Task 5. Find optimum collision energy for MRM acquisition 35

With this exercise, you learn how to determine the best Agilent Triple Quad LC/MS acquisition settings for analyzing your compounds of interest. These instructions help you understand not only how to set up a worklist to optimize instrument parameters for best sensitivity in acquisition, but also how to use the Agilent MassHunter Qualitative Analysis program to identify parameter values producing optimum signal response.

In this exercise, you create an acquisition method for a mixture of four sulfa drugs, optimizing both the fragmentor and collision energy voltages to maximize sensitivity. One of the ways to optimize parameters is to create a worklist, or sequence, of data file acquisitions, each using a different method. This exercise uses this protocol for method development.

Another way to develop a method is to use the manual tune capability to optimize various parameters, including collision energy, to obtain the optimal signal response for each multiple reaction monitoring (MRM) transition. A third technique has you set up an acquisition method that directs the instrument to make multiple injections of the sample from an autosampler vial and acquire the data to a single data file. The method contains multiple time segments, one for each injection, with an incremental change made to a particular parameter (e.g., collision energy) in each segment.



This exercise uses the first protocol for method development.

NOTE

See the *Agilent Triple Quad LC/MS Concepts Guide* to learn more about how the triple quadrupole mass spectrometer works and why the fragmentor and collision energy voltages are important. For background information, see Chapter 3, Agilent Triple Quad MS and Sensitivity, in the *Concepts Guide*. See the Online Help for detailed information on how the program works.

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.

Before you begin...

For this exercise you analyze a mixture of four sulfonamide compounds.

If you do not intend to perform data acquisition but want to learn how to use the Qualitative Analysis software for method development, you can skip this section, which gives instructions on how to prepare the demo sample. You then perform only those instructions that show you how to use the Qualitative Analysis software with the sulfa drug data files shipped with the system.

The Electrospray LC Demo Sample (P/N 59987-20033) contains five ampoules with 100 ng/ μ L each of sulfamethizole (M+H)⁺ = 271, sulfamethazine (M+H)⁺ = 279, sulfachloropyridazine (M+H)⁺ = 285, and sulfadimethoxine (M+H)⁺ = 311.



NOTE

Determining optimal parameter values for acquiring sample compound data requires that the Agilent Triple Quad instrument already be tuned on the Tuning Mix calibrant ions. Before proceeding with this exercise, make sure you have used Checktune or Autotune to verify that calibrant ions each have the proper mass assignment, peak width, and signal intensity.

See the Agilent Triple Quad LC/MS Quick Start Guide or Online Help for instructions on tuning the instrument.

1 Prepare the LC solvent.

In 1-liter reservoirs of HPLC-grade water and acetonitrile (ACN), add 1 ml of $5M NH_4HCO_2$ (Ammonium Formate) each to make $5mM NH_4HCO_2$ in H2O and ACN and use for the A and B channels, respectively.

- **2** Prepare the sample.
 - **a** Add 10 μL sulfa mix from one of the ampoules (500 μL) to 990 μL of solvent A in an Eppendorf vial so that the final concentration is 1 ng/μL.
 - **b** Place a sample vial containing an injectable amount of the prepared sample in the autosampler.
- **3** Set up the LC column.

Use this Agilent column or equivalent: Zorbax, SB-C18 2.1mm x 30mm, 3.5um, 100Å, p/n 873700-902.

4 Set the column temperature.

Agilent suggests a column temperature of 40° C, but this exercise can run at room temperature.

5 Copy the data files to a directory on your PC.

Copy the folder named **SulfaDrugs** in the **Data** folder on your software CD to any location on your hard disk. This folder contains all the data files needed for this exercise.

NOTE

Do not re-use the sulfa drug data files already on your system unless you know that you copied them from the originals on the CD and you are the only one using them.

Do not use these sample data files to look at sample information or print a report.

Task 1. Enter acquisition parameters and acquire data

In this exercise, you enter the conditions for the analysis of the sulfa drug mix.

Steps		De	Detailed Instructions		Comments	
1 Enter for s See	er LC parameters appropriate sulfa drug mix. e Table 1.	a b c	Double-click the Agilent Data Acquisition icon. Make sure that Acquisition appears as the selection in the Context text box. If Tune is the selection, select Acquisition from the Context dropdown menu. Enter the LC parameters listed in the Table 1.	•	The Agilent MassHunter Workstation Data Acquisition window appears. See Figure 1.	

Table 1 LC parameters for sulfa drug mix

Parameter	Value		
PUMP			
• Flowrate	800 µL/min		
Solvent A	$5 \text{ mM NH}_4\text{HCO}_2$ in H ₂ O		
Solvent B	5 mM NH ₄ HCO ₂ in ACN		
• Gradient (min - %B)	0 min - 13% 1.80 min - 60% 2.50 min - 60%		
• Stop Time	2.50 min		
Post Time	2.50 min		
INJECTOR			
• Inj. Vol.	1 µL		
Injection	Standard		
Draw Position	3.0 mm		
UV DETECTOR			
• Ch A	254 nm (4 nm BW on DAD)		

Task 1. Enter acquisition parameters and acquire data

Table 1 LC parameters for sulfa drug mix

Parameter	Value
• REF A (DAD only)	400 nm (80 nm BW)
COL THERM	
• Temp	40°C



Figure 1 Agilent MassHunter Workstation Data Acquisition window

Task 1. Enter acquisition parameters and acquire data

Steps		De	etailed Instructions	Comments
2	Enter MS parameters appropriate for sulfa drug mix and save the method as <i>iii</i> MS2Scantest.m, where <i>iii</i> are your initials.	a b c	Click the MS QQQ tab. Click the Scan Type cell, and select MS2Scan from the list. Enter the other MS parameters as listed in Table 2. These parameters are in either the Acquisition or the Source	
		d	tabs. Save the method as <i>iii</i> MS2Scantest.m , where <i>iii</i> are your initials.	

Table 2MS parameters for sulfa drug mix

Parameter	Value
• Source	ESI (+ polarity)
• Gas Temp	350°C
• Scan Type	MS2Scan
• Nebulizer	50 psi
• Dry Gas	12 L/min
• Range	100 to 400

Ion source Stop time ESI C No imit/As Pump Tune file Time file Time file Tune file Time file Time file Time file C File Time file C Time file Time file C File Time file C Time file Time file C File # Time Scan Type Ton Polarly Div Valve Delta EMV Stored V 0 C	Acquisition Source Chromatogram Instrument Diagnostics Scen segments Segment Name Start Mass End Mass Scan Time Fragmentor Image: Segment Name Start Mass End Mass Scan Time Fragmentor Image: Segment Name Start Mass Scan Time Fragmentor Image: Segment Name Start Mass Scan Time Fragmentor Image: Segment Name Start Mass Scan Time Fragmentor	Apply Reset
M62 Scan MFM Product Ion Precurso Ion Neutral Cash Neutral Gain	Scan parameters Step rize: 0.1 anu Data storage: Profile Threshold 0	

Figure 2 Select Scan Type of MS2 Scan in the Acquisition tab

Task 1. Enter acquisition parameters and acquire data

Steps	Detailed Instructions	Comments		
 3 Acquire data (optional). Set up a one-line worklist with the method you just created. Name the data file <i>iiisulfamix01.d</i>, where <i>iii</i> are your initials. Designate a directory path to hold your data files and method. 	 a If necessary, click the Worklist icon to bring up the worklist. b Select Worklist > Worklist Run Parameters. Verify that the parameters are set properly. Click OK. c Type <i>iii</i>MS2Scantest.m and <i>iii</i>sulfamix01.d as the method name and data file name, respectively. d Click OK. e At the bottom left portion of the Acquisition window, where you can find the worklist, mark the check box to the left of the sample as shown below. 	 You have just acquired a full scan MS data file to see what ions are being formed from the sample. This step is optional because you can perform the next step with an example data file that comes with the software. If you prefer, you can create your own data file as described in this step. 		
	t Click the Kun icon.			

Task 2. Determine precursor ion masses

In this exercise, you determine the precursor ions for each of the sulfa drugs in the acquired data file.

Steps		Detailed Instructions		C	Comments	
1	Open the acquired data file. • In the Agilent MassHunter Qualitative Analysis program, open either the example file, sulfamix01.d , or the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 11.	a	Double-click the Agilent MassHunter Qualitative Analysis icon. The system displays the Open Data File dialog box.	•	When you open the sulfa drug directory after installation, the Load result data (lower left corner) check box is grayed out. If you see the check box marked, this means that the data file(s) already contains results. <i>Clear this</i> <i>check box before opening the file</i> .	

Open Data File						? 🛛
Look jn:	🗀 QualData			•	(-	
My Recent Documents Desktop My Documents My Computer	Sulfa_SIM60 Sulfa_SIM80 Sulfa_SIM80 Sulfa_SIM100 Sulfa_SIM100 Sulfa_SIM180 SulfamixO1.d SulfamixMRM SulfamixMRM SulfamixMRM SulfamixMRM SulfamixMRM	1 (1) 1 (1) .d (1) .d .d .d .d .d .d .d .10.d .15.d .20.d .20.d .20.d .30.d	Sulfamix/MRM_35.d Sulfamix/PI_15.d Sulfamix/PI_30.d			
My Network Places	Filenames : Files of type :	Data File	es (*.d)		•	Open Cancel
Options C Load worklis C Load results Use current Load result Load result Run 'File Op selected me	at method ; method method data pen' actions from thod		-Sample Informatio Sample Name : User Name : Sample Position : Description :	n		

Task 2. Determine precursor ion masses

Steps	Detailed Instructions	Comments
	 b Do one of the following: Select the example data file sulfamix01.d, and click Open. Select the data file you created in "Task 1. Enter acquisition parameters and acquire data" on page 11, and click Open. By default, the system displays the Total Ion Chromatogram (TIC). 	 The figure below shows the default layout. This is what you want to see. The Qualitative Analysis program displays a newly opened data file with the same layout and display settings used for the previous data file. Therefore, you MUST make sure to return to the default settings for this exercise.
	S Applent Massifunter Qualitative Analysis - Default.m	
Refore you begin makes	Elle Edit Yew Compounds Chromatograms Spectra Method Actions Icol	s Help

Before you begin, make sure that all previous settings are returned to their default values:

- Restore default layouts
 - Click View, and select Window Layouts.
 - Select Restore Default Layout.
- Make sure the method is default.m. (see title bar)
 - Select **Method > Open**.
 - Select default.m, and click
 Open.
- Return display options to default settings.
 - Select Tools > Plot Display Options...
 - Click **Default**, and then **OK**.



Task 2. Determine precursor ion masses

Steps				etailed Instructions	Comments	
 2 Determine precur for all four peaks. You have deter correctly if you are similar to the this table: 	rsor ion m mined the find the v hose show	asses m alues /n in	 a In the Chromatogram Results window, make sure that the Range Select icon in the toolbar → is On. b Click the left mouse button and drag the cursor across the first peak to produce a shaded region, as in the 		 The system displays an averaged spectrum across the peak in the Spectrum Preview window. The precursor mass of the first compound, sulfamethizole, is determined to be m/z 270.9. 	
Compound	RT	m/z	C	Right-click inside the shaded region,	click the apex of the peak.	
Sulfamethizole	0.47	270.9	-	and select Extract Spectrum from the		

Compound		
Sulfamethizole	0.47	270.9
Sulfachloropyridazine	0.88	284.9
Sulfamethazine	1.20	279.0
Sulfadimethoxine	2.23	311.0

Close the data file after finding • the precursor ion masses.





d Repeat step a through step c for the other compounds. The precursor ion masses should

match those in the table in step 2.

- e Select File > Close Data File.
- f When asked if you want to save the results, click No.
- · Some compounds form sodium (Na) and/or potassium (K) adducts as well, corresponding to M + 23 and M + 39 masses respectively. Seeing these masses along with the M + H can make for an easy confirmation of which ion is the pseudo-molecular ion (M + H)+.

Task 3. Find optimum fragmentor voltage for maximum response

Task 3 shows you how to carry out the optimization for fragmentor voltage by creating selected ion-monitoring experiments for each compound within a method and setting up multiple methods with varying fragmentor voltages.

Steps	Detailed Instructions	Comments	
 Set up six methods for six different fragmentor voltages. Change to a SIM experiment. Use 60, 80, 100 and 140, 180 and 220 volts as the fragmentor voltages for the six methods. Save the methods as <i>iii</i>MS2SIM<i>xxx.m</i>, where <i>iii</i> are your initials and <i>xxx</i> is the voltage. 	a In the Scan Type dropdow MS2SIM. Sample Properties WPS Bin F Ion source MMI MI Tune file atunes.TUNE XML 6d Time segments # Time Scan Type Ion Mode 1 0 MBM ESI 2 MS2 Scan Product Ion Precursor Ion Neutral Loss Neutral Cos Neutral Cos	n list, select	

Steps	De	etailed Instructions In the Acquisition tab, enter the Compound Name and Mass (precursor ion mass) for sulfadimethoxine. Click the arrow to the left of the compound name, and select Add Row. Enter Compound Name and Mass for sulfachloropyridazine. Repeat steps c and d for sulfamethazine and sulfamethizole. Save the method as <i>iii</i> MS2SIM140.m, where <i>iii</i> are your initials. Change the fragmentor voltage to 60, and save the method as <i>iii</i> MS2SIM060, where <i>iii</i> are your initials. Repeat step g for voltages 80, 100, 180 and 220, saving the methods as <i>iii</i> MS2SIM080, <i>iii</i> MS2SIM100, <i>iii</i> MS2SIM180 and <i>iii</i> MS2SIM220, where <i>iii</i> are your initials.			Co	Comments			
	b c d e f g				• • • • • • • • • • • • • • • • • • •	With the MS different set the Acquisit The Instrum Acquisition experiment mass, startin fragmentor v example bel	S2SIM Scan of columns ion window ent Control program cre for each con ng with a de voltage of 1 ow.	Type set, a s appears in n. and Data eates a SIM mpound efault 40. See the	
	Acquisition Source Chromatogram Diagnostics								
	Г	Scan segments							
		Compound Name	ISTD?	Mass 🗸	MS2 Res	Dwell	Fragmentor		
		sulfadimethoxine		311	Unit	200	140		
				1 2851	Unit	1 200	140		
		sultachloropyridazine		200	OTIK				
		sulfachloropyridazine sulfamethazine		203	Unit	200	140		

Task 3. Find optimum fragmentor voltage for maximum response

Steps		Detailed Instructions				C	Comments			
2 Sı (c	et up and run the worklist optional). Set up six samples with Sample Name SulfaDrugMix to inject 1 ul from vials 1-6 or the ones you choose. Specify the data files as <i>iii</i> SulfaSIMxxx.d, where <i>iii</i> are your initials and <i>xxx</i> is the voltage.	a b c d e f g h	Click make Ente volta Righ work Sam Add for v To se left o Para Click locat click Marl Sam	the Worklis e sure the wo r the informa- ige run. t-click the up dist, and sele ples . five more sau oltages 80-22 et up the run, corner, and se imeters . r the paths for files. t the tab to sp tions, specify OK . < the checkbo ple Name for ples.	t icon if necess orklist is visible. Ition for the 60 oper left corner of ect Add Multiple mples to the wo 20, and click OK right-click the f elect Worklist F or the method a pecify the samp the locations a ox to the left of r each of the six	ary to • of the e orklist upper Run nd le vial ind	This step is opti can use data file system to perfo tasks in this exe	ional because you es shipped with the rm many of the ercise.		
				Cample Name	Cample Desition	Asg Math	nd Data File	Cample Tupe		
			1 4	SulfaDrugMix	Vial 1	MS2SIM060	m dt\Sulfa SIM060 d	Sample		
		-	2 4	SulfaDrugMix	Vial 1	MS2SIM080	m d:\Sulfa_SIM080.d	Sample		
			3 ¥	SulfaDrugMix	Vial 1	MS2SIM100	m d:\Sulfa SIM100.d	Sample		
			4 ¥	SulfaDrugMix	Vial 1	MS2SIM140	m d:\Sulfa_SIM140.d	Sample		
			5 ¥	SulfaDrugMix	Vial 1	MS2SIM180	m d:\Sulfa_SIM180.d	Sample		
			6 ¥	SulfaDrugMix	Vial 1	MS2SIM220	m d:\Sulfa_SIM220.d	Sample		
		i	Sele	ct Run > Wo	rklist.	•	Note that the p	rogram only runs		
							those samples t	that are enabled		

with a checkmark.

Task 3. Find optimum fragmentor voltage for maximum response

Steps	Detailed Instructions	Comments			
 3 Set up a qualitative method to v the EIC data automatically. Open the data file Sulfa_SIM60.d or your own <i>iii</i>Sulfa_SIM60.d, where <i>iii</i> a your initials. In the Method Editor, add in t EICs corresponding to the precursor ion masses of 271, 279, 285, and 311. 	 a Select File > Open Date The system displays the File dialog box b Select either Sulfa_SIIF b Select either Sulfa_SIIF b Sulfa_SIM60.d, and 	ta File. • The Qualitative Analysis program te Open Data should be open. If not, see step 1 of Task 1 in this exercise. Task 1 in this exercise. V60.d or click Open. click Open. should be open. If not, see step 1 of Task 1 in this exercise. sectors V60.d or click Open. sectors sectors Sectors the the the file sectors the the the file sectors to the the the file sectors to the the the file sectors to the the the file			
 Save the method as <i>iii</i>Exercis where <i>"iii"</i> are your initials. 	se1, © Ber Chronologians <u>BAN Brite Secto</u> Der Spectra <u>Backgrounds</u> Conscounds	12- 1- 0.5- 0.6- 0.4- 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.5 0.9 1. 11 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2 21 22 2.3 2.4 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.5 0.9 1. 11 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2 21 22 2.3 2.4			
		Method Editor: Define Chromatograms			
	Method Explorer: Default.m	▲ [] · · · · · · () Method Items • []]			
	Chromatogram	Defined chrometograms			
	Integrate and Extract Peak Spectra Extract Peak Spectran Smooth Exclude Mass(en) Calculate Signal to Noise Define Chromatograms Adjust Delay Time Compounds Compounds General Worklist Automation	BBC (al) AI (Syste-summed) Add Chromatodram definition Change Type: BrC Extended MS Chromatogram Advanced Excluded Masses MS Weet AII Scots AI scan types Image and the second and the s			

c Select Method > View Method Editor. The system displays the Method Editor window. (See figure on next page.)

Steps	Detailed Instructions	Comments
	d If necessary, select Define Chromatograms from the Method Items pull-down menu.	 The default Method Editor list selection after installation is Integrate.
	🖻 Method Editor: Define Chromatograms	
	🛃 🖉 🕶 🖓 🔹 💽 🕴 Method Items 🔹 🚑 📑	
	Defined chromatograms	
	BPC (all) All (Cycle-summed)	Add Change Delete
	Chromatogram definition	
	Type: BPC	ate when cted
	MS Chromatogram Advanced Excluded Mass	ses
	MS level: All v Scans: All scan types	✓
	Polarity: Both 🔹 m/z of interest: Ar	ny 🗸
	m/z value(s):	
	☑ Do cycle sum	
	e To delete the BPC chromatogram, click	
	Delete . f For the Chromatogram Definition Type	
	select EIC .	
	g In the MS Chromatogram tab, make	
	sure MS Level is set to All and Scans is set to All Scan Tynes	
	h Clear the Do cycle sum check box.	
	i Enter 271 as the m/z value .	
	J Ulick Add. k Bepeat steps i and i for the other	
	precursor ions, 279, 285 and 311.	
	I Select Method > Save As.	
	box.	
	m Save the method as <i>iii</i> Exercise 1.m.	

Steps	Detailed Instructions	Comments
 4 Extract the chromatogram for the data file and view the results. Make sure you can see all five chromatograms, the TIC and four 	 a To apply the method settings to the data file, click the Extract Defined Chromatogram icon on the toolbar. 	
EICs.	Method Editor: Define Chromatograms	
	 b To see the TIC and four EICs, click the down arrow to display a numerical list as shown in the example below. c Select 5 to view five chromatograms simultaneously. The system displays chromatogram results as shown below. 	
	Δ Chromatogram Results	×
	2 ↔ \$ • <td>% Minutes 1 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 Acquisition Time (min) 1 1 1 1 1 1 1 1</td>	% Minutes 1 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 Acquisition Time (min) 1 1 1 1 1 1 1 1

Steps	Detailed Instructions	Comments			
 5 Extract the remaining ion chromatograms automatically. • Extract Defined Chromatograms should be the default action for Assign File Open Actions. • Open the remaining data files, 	 a Select Assign File Open Actions from the Method Editor dropdown menu. b Make sure that Actions to be run is set to Extract Defined Chromatograms. Method Editor: Assign Actions on Opening a 	 The Qualitative Analysis Method Editor lets you define actions to be performed automatically upon opening a data file(s). 			
Sulfa_SIM80.d through Sulfa_SIM220.d. • Close the Method Explorer.	Available actions Extract Defined Chromatograms Integrate And Extract Peak Spectra Find Compounds by Auto MS/MS Find Compounds by Targeted MS/MS Find Compounds by Molecular Feature Export mzData Generate Compound Empirical Formula Integrate Chromatograms Extract Peak Spectra Actions to be run				
	 c Select File > Open Data File. The system displays the Open Data File dialog box. d Select all the data files to be opened. e Mark the Run 'File Open' actions from selected method checkbox. (lower left corner) 				

Task 3. Find optimum fragmentor voltage for maximum response

Detailed Instructions	Comments		
Open Data File	?		
Uppen Data Frie Look in: SulfaDrugs Sulfa_SIM60.d Sulfa_SIM60.d Sulfa_SIM20.d SulfamM6M8.15.d SulfamMM8.136.d SulfamMM8.35.d SulfamMM8.136.d SulfamM8M.35.d SulfamM8.136.d SulfamM8.136.d SulfamM8.136.d SulfamM8.136.d			
My Network Places Filenames : "Sulfa	SIM60.d" " Sulfa_SIM80.d" " Sulfa_SIM100.d 💌 Open		
Files of type : Data F	iles (*.d) Cancel		
	Help		
Options C Load worklist method C Load results method Use current method Ded Load result data	ample Information ample Name : ser Name : ample Position : escription :		

The Qualitative Analysis program displays all the EICs for all the data files selected.

g To close the Method Explorer and Method Editor, click the **X** in the upper right corner of each window.



Steps		Detailed Instructions	Comments			
6	Select the fragmentor voltage that produces the maximum response for each of the precursor ions. Close the data files after you determine the optimum voltage.	 a In the Data Navigator window, highlight the EICs for 271.0 m/z. b Click the Show only the highlighted items icon, . Only the 271 m/z check boxes are now marked. 	 You press the Ctrl key to be able to select multiple files from the list You press the Shift key to be able to select a group of files. A fragmentor voltage of 100 should be sufficient for each precursor ion. You can now determine the product ions that are available for the multiple-reaction monitoring experiments to maximize sensitivity for the analysis. 			
		다 2013년 Handhort Guillithe Andyla, service) an De De See Overdagens Servic Both State Service 2013년 1월 2017년 1월 2014년 2018년 1월 2014년 1월 2014				



- c Look at the relative intensities of each peak to determine which fragmentor voltage setting will be best to use for the 271 precursor.
 Or to overlay the peaks, you can click **Overlaid Mode**.
- **d** Repeat steps a-c for the other three base peaks or precursor ions.
- e Select File > Close Data File.
- f Click **Close** when the Close Data File dialog box appears.

Task 4. Determine product ion masses

Task 4. Determine product ion masses

In this part of the method development, we will use three collision energies to determine the best fragment ions to use for the eventual Multiple Reaction Monitoring (MRMs).

 1 Set up three product ion acquisition methods and acquire data. a Click the MS QQQ tab. b Select Production in the Scan Type field to scan each precursor ion for all its product ions. c Enter all MS parameters as listed in the example below, but change the Fragmentor voltage to the optimum voltage you determined in the previous task. c Save methods as <i>iiiSulfamix</i> PI_xx.m, where <i>iii</i> are your initials and xx is the collision energy. c Repeat step c and step d for collision energies of 30 and 45. 	St	eps	Detai	led Instructions			C	omment	ts		
Acquisition Source Chromatogram Instrument Diagnostics ESI No limit/As Pump I min file min file file	1	 Set up three product ion acquisition methods and acquire data. Use the MS parameters in the example below, but change the Fragmentor voltage to the optimum voltage you determined in the previous task. Save methods as <i>iii</i>Sulfamix PI_xx.m, where <i>iii</i> are your initials and xx is the collision energy. 	a Cli b Se fie its c En the Co Fra op d Sa PI e Re en	ck the MS QQQ ta lect Production Id to scan each product ions. Iter all MS parame e example below, Illision Energy is s agmentor voltage timum voltage de ive the method as _15.m. epeat step c and si ivergies of 30 and 4	ab. in the So recursor eters as I making s set to 15 is set to terminec <i>iii</i> Sulfa tep d for 5.	an Type ion for a isted in sure the and th the in Task nix collisio	e all e c c 3. n				
# Time Scan Type Ion Polarity Div Valve Delta EMV Stored Ivp 1 0 Product Ion Positive To MS 0 Image: Scan parameters Step size: 0.1 amu Data storage: Profile		Ion source Stop time ESI ▼ ESI Tune file • No limit/As Pump atures.tune.sml jatures.tune.sml Image: transformation of tra	min	Acquisition Source Chr Scan segments Segment Name I sulfadmethxxine sulfadmethxxine I sulfadmethxxine sulfadmethxxine I sulfadmethxxine sulfadmethxxine I sulfadmethxxine sulfadmethxxine I Scan parameters Staff parameters Staff parameters Step size: 0.1 Data storage: Prof	romatogram in Precursor Ion V 311 285 279 271 271	strument Di MS2 From 50 50 50 50 80 80 80 80 80 80 80 80 80 80 80 80 80	Agnostics MS2 To 320 320 320 320	Scan Time 250 250 250 250	Fragmentor 140 140 140 140	Collision Energy 15 15 15 15 15	

- 2 Set up and run the worklist (optional).
 - Specify the data files as iiiSulfamix Pl xx.d, where iii are your initials and xx is the collision energy.
- a Scroll down if necessary to make sure the worklist is visible.
- **b** Add three samples to the worklist for collision energies 15, 30 and 45.
- c Mark the checkbox to the left of the Sample Name for each sample you are adding.
- d Select Run > Worklist.

- · This step is optional because you can determine the product ion masses from the data files shipped with the system.
- · Use the instructions in Step 2 of Task 3 to set up the worklist.

-

Integrate when extracted

311.0

~

Scans: Production

Precursor ion m/z:

Delete

*

~

Task 4. Determine product ion masses

Steps		Detailed Instructions		Comments		
St 3	 eps Set up a qualitative method to integrate and extract product ion spectra. Use the data files SulfamixPl_xx.d, where xx is the collision energy, or your own data files, <i>iii</i>SulfamixPl_xx.d. Open Method Explorer and Method Editor. Use TICs set up for MS/MS, product ion and each of the precursor ions 271, 279, 285, 311. Make sure the MS/MS integrator has been selected and the maximum number of peaks has been limited to the largest 100 peaks. Add the ability to integrate and extract peak spectra to the file actions run upon data opening. Save the changes to the current 	a b c d f g h i j k	Click the Open Data File icon in the toolbar. Select SulfamixPl_15.d. Make sure that the Run File Open Actions from Specified Method check box is clear, and click Open. Make sure the Method Explorer and the Method Editor windows are displayed; otherwise, click the Method Explorer and then Method Editor icons. Ounder Chromatograms in Method Explorer, click Define Chromatograms. Delete any existing chromatograms box. Select TIC from the Chromatogram Definition list. For MS Level, select MS/MS. For Scans, select Product ion. For Precursor ion m/z, enter 271. Click Add.		The Qualitative Analysis program should already be open and contain <i>iii</i> exercise 1.m as the method.	
	method.	I	Repeat steps j and k for each ion.			
		1	Method Editor: Define Chromatograms			
		:	Define Chromatograms Define Chromatograms Defined Chromatograms TiC Prod (271.0 m/2) (Cycle-summed) TiC Prod (280.0 m/2) (Cycle-summed) TiC Prod (280.0 m/2) (Cycle-summed) TiC Prod (281.0 m/2) (Cycle-summed)	•	Add Change	

Chromatogram Definition

MS level: MS/MS

Both

MS Chromatogram Advanced Excluded Masses

~

Type: TIC

Polarity:

m/z value(s):

🔽 Do cycle sum

Steps	Detailed Instructions	Comments
	 m From the Method Editor drop-down list, select Integrate. n Select the MS/MS Integrator, if necessary. 	
	🚮 💽 Integrate 🔹 🕒 📳	
	Integrator Peak Filters Integrator General Integrator Otelector Start threshold: Point sampling: 1 Stop threshold: 00 Filtering: 5 point Peak location: Top Baseline Allocation: 5 If either edge < 100 Click the Peak Filters tab. Make sure	
	that the Limit to the largest check box is marked and set to the value 100 (peaks) as shown below.	
	🚮 💽 Integrate 🗾 🖉	
	Integrator Peak Filters Peak qualification Retain peaks for which Peak area >= 1.000 % of largest	peak V
	 Click General in Method Explorer, and then click Assign File Open Actions. Select Integrate and extract peak spectra from the Available actions list and click to add this to Actions to be run. 	

Steps	Detailed Instructions	Comments
	Method Editor: Assign Actions on Opening a	a Data File
	🚦 🚮 🕟 Assign File Open Actions	- 🕒 🐚
	Available actions	
	Extract Defined Chromatograms Integrate And Extract Peak Spectra Integrate Chromatograms Extract Peak Spectra Print Qualitative Analysis Report	
	Actions to be run Extract Defined Chromatograms Integrate And Extract Peak Spectra	
		▲▼▼
	 To apply the changes to the cur method, <i>iii</i>exercise1.m, click the Method icon. 	rrent e Save
4 Run the qualitative method on the current data file.	 In the Method Editor: Assign Ac on Opening a Data File window, the Action button. 	 The Qual method first extracts the product ion chromatograms for each precursor ion in the data file. Next, it finds the largest peak in the total ion chromatograms, and integrates and extracts peak spectra from under each integrated peak. See Figure 3 on page 32 on the next page.



Figure 3 Results for integration and extraction of peak spectra.

Steps Detailed Instructions		Comments		
 5 Run the qualitative method on the remaining product ion data files. • Use either the example files, Sulfamix Pl_xx.d, or the data files you acquired in step 2. 	 a Select File > Open Data File. The system displays the Open Data File dialog box. b Hold the Ctrl key and do one of these: Select the two data files Sulfamix PI_30.d, and Sulfamix PI_45.d. Select the data files you acquired in step 2. c Mark the Run actions from method checkbox in the Open Data File dialog box, and click Open. 	 After the data files open, the Qual method first extracts the product ion chromatograms for each precursor ion. Next, it finds the largest peak in the total ion chromatograms, and integrates and extracts peak spectra from under each integrated peak. See the figure in step 6. 		

Steps	Detailed Instructions	Comments			
 6 Identify product ions. View each set of TICs and spectra individually (e.g., 271 m/z first). Close the data files. 	 a In the Data Navigator, select the TICs and spectra for the 271 m/z precursor ion. b Click the Show only the highlighted items icon,	 The m/z 155.7 product ion is the most abundant of any product ion and the highest signal is recorded at 15 V. This means that a good choice for the MRM for sulfamethizole would be 271.0 > 155.7 when the collision energy is around 15 V. 			



- e Click the Close Data File icon, and click Close when the dialog box containing the list of data files pops up.
- The product ions appear to be: Sulfamethizole-271.0 > 155.7 Sulfamethazine-279.0 > 185.8 Sulfachloropyridazine-285.0 > 155.7 Sultadimethozine-311 > 155.7

Task 5. Find optimum collision energy for MRM acquisition

In this task, you set up MRM acquisition methods for the sulfa drugs for different collision energies. By examining the spectra and comparing peak intensities, you determine the optimal collision energy settings for the compounds.

Steps	Detailed Instructions	Comments			
 Set up three MRM acquisition methods. Use all the MS parameters in the example below except for the collision energy value. Use collision energies of 10, 15 and 20. Save methods as <i>iii</i>Sulfamix MRM_xx.m, where <i>iii</i> are your initials and xx is the collision energy. 	 a Click the MS QQQ tab. b Set Scan Type to MRM. c Enter all MS parameters shown in the example below except for the collision energy value. d In the collision energy column, type 10 for each compound. e Save the method as <i>iii</i>Sulfamix MRM_10.m. f Repeat step d and step e for collision energies of 15, 20, saving the methods as <i>iii</i>Sulfamix MRM_xx.m, where <i>iii</i> are your initials and xx is the collision energy. 	 Because the largest peaks were produced with a collision energy of 15 in the previous exercise, you will look at only those collision energies to either side of 15. 			

Sample Properties WPS Bin Pump Column DAD MS QQQ										
ESI ESI No lime	Ao	quisition Source Chro	matogram	Diagnostics						
C 1 min		Compound Name	ISTD?	Precursor Ion 🗸	MS1 Res	Product Ion ∇	MS2 Res	Dwell	Fragmentor	Collision Energy
Time filtering		sulfamethoxine		311	Unit	155.7	Unit	50	100	35
Peak width 0.07 mm		sulfachloropyridazine		285	Unit	155.7	Unit	50	100	35
Time segments		sulfamethazine		279	Unit	185.7	Unit	50	100	35
# Time Scan Type Ion Polarity Diverter Valve Delta EMV Data Stored		sulfamethizole		271	Unit	155.7	Unit	50	100	35
▶ 1 0 MRM Positive To MS 0 4.67 cycles/s 214.0 ms/cycle										

Task 5. Find optimum collision energy for MRM acquisition

Steps	Detailed Instructions	Comments				
 2 Set up and run the worklist (optional). Specify the data files as <i>iii</i>Sulfamix MRM_xx.d, where <i>iii</i> are your initials and xx is the collision energy. 	 a Click the Worklist icon if necessary to make sure the worklist is visible. b Add three samples to the worklist for collision energies 10, 15, 20. c Mark the checkbox to the left of the Sample Name for each of the three samples. d Select Run > Worklist. 	 This step is optional because you can use the six example data files in the next step. 				
 3 Compare the compound transition intensities at different collision energies. Open all the MRM data files. Set the MRM chromatogram extraction parameters as shown at right for all transitions. Disable the TICs for clarity and examine the peak intensities. Compare the intensities of each compound transition obtained at one collision energy with the same compound transition obtained at another collision energy. (Do this in Overlaid Mode with all the MRM chromatograms.) 	 a Open the Qualitative Analysis software. b Clear the Run 'File Open' actions check box. c Open all the MRM data files in the Qualitative Analysis program. d Right-click the Chromatogram Results window, and select Extract Chromatograms from the shortcut menu. e To select all data files, click the last file while holding down the Shift key. f Enter the parameters as listed in the example below, and click OK. g Clear the TIC check boxes to make the MRM chromatograms easier to view. 	• Why a spectrum for MRM? It's a feature of the program to show spectra even for MRM experiments and can be quite handy for comparing relative intensities of product ions generated from the same precursor.				
 Close the data files but don't 	Extract Chromatograms	×				
 save results. Refer to Table 3 on page 38 for optimal method settings for each compound. 	List of opened data files SulfamisMRM_10.d SulfamisMRM_15.d SulfamisMRM_20.d MS Chromatogram Ac MS levet: MS/MS	Integrate when extracted dvanced Excluded Masses Scans: Multiple reaction monitor				

Polarity:

Positive

Transition: All

~

Cancel

ΟK
Develop an acquisition method for the Agilent Triple Quad LC/MS 1

Task 5. Find optimum collision energy for MRM acquisition

teps	Detai	led Instructions	Comments
	h Cli i Co coi in t	ck the Overlaid Mode icon, A mpare peak intensities for each mpound transition in each data the Chromatogram Results win	Compare the colors shown in Chromatogram Results with the file color next to the MRM transition dow. name in Navigator.
	🌺 Data Navigator	× A Chromatogram Results	
	Sort by Date File Sufficient MIMI, 10 d Sufficient MIMI, 20 d Suf	N N	

Unless you decide to acquire MRMs at lower collision energies, you should find that the optimal method settings are as shown in Table 3.

- j Click the Close Data File icon, and click Close when the Close Data File dialog box appears.
- You now have all the information you need to do an MRM acquisition experiment of the sulfa drug mixture. Consider doing at least one more run with those settings.

-

1 Develop an acquisition method for the Agilent Triple Quad LC/MS

Task 5. Find optimum collision energy for MRM acquisition

Table 3

Compound Energy	MRM Transition	Fragmentor	Collision
Sulfamethizole	271.0 > 155.8	100 V	10
Sulfamethazine	279.0 > 185.7	100	15
Sulfachloropyradizine	285.0 > 155.7	100	10
Sulfadimethoxine	311.0 > 155.7	100	20



Agilent 6410 Triple Quad LC/MS Familiarization Guide

Exercise 2 Set up and quantitate a batch of acquired data files

Task 1. Set up a new batch41Task 2. Set up a new method for the batch44Task 3. Set up target compounds47Task 4. Set up quantitation50Task 5. Analyze and save the batch56

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 on your software CD 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.
 - Enter the concentration of the highest concentration calibration standard and the dilution pattern.
 - Set up qualifier ions and the calibration curve.
- Automatically quantitate the batch and save the results.



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.

Before you begin...

Make sure that you have copied the **DrugsOfAbuse** folder from the **Data** folder of the software CD to a folder on your system.

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.

Steps		Detailed Instructions		C	Comments	
1	 Create a new batch to hold samples. Select all of the data files from the DrugsOfAbuse folder. Name the batch file, <i>iii_test_01</i>, where "<i>iii</i>" are your initials. 	a	To start the Quantitative Analysis program, click the Quantitative Analysis icon on your Desktop. When you first use the program, the default layout appears, as shown in Figure 4 below.	•	You can also access the program by selecting Programs > Agilent > MassHunter Workstation > Quantitative Analysis from the Start menu.	

Agilent MassHunter Quantitative Analysis						
ie <u>E</u> dit <u>V</u> iew <u>A</u> nalyze <u>M</u> ethod <u>U</u> pdate <u>R</u> eport <u>T</u> ools <u>H</u> elp						
🗁 😡 🖓 🖓 🥵 Analyze Batch 🕖 🕴 Layout: 🔛 🔢 🕅 🛄 🗛 🧭 Restore Default Layout						
itch Table						
ample: 🝸 👢 Sample Type: 💌 Compound: 💷	ISTD:	Tim			•	****
Sample						
V Name Type Level Acq. Date-Time						
npound Information	Calibration Curve					
npound Information : 引 ● ● ▲ 五 山 会 山 : マ ↔ キ 企	Calibration Curve	er Origi	nı	♥ Weight:		ISTD QC
npound Information ミーー・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・・	Calibration Curve	Crigi	int	Veight:		LSTD QC
npound Information	Celibration Curve	e Origi	in:	e Weight:		ISTD QC
npound Information ●●●●▲元元此会↓↓↓ ▼ ◆ 企 metogram 0 1 0 9- 0 8-	Calibration Curve Image: I	a Origi	n:	Veight		ISTD QC
npound Information :: ■ ● ● ▲ 杰 :: nstogram 1 3- 2- 2-	Calibration Curve □ □ □ □ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓	Crig		Weight		ISTD QC
npound Information :	 Calibration Curve ● ● ↓ Tryoni ● ▼ ↓ ‡ 英 • ● ×10² ● ×10² ● ×10² ● ×10² ● ×10² ● ×10² 	Z Origi		Weight		ISTD QC
npound Information :	Calibration Curve Image: I	Crigi		Weight		ISTD QC
npound Information : E ● ● 八 五 山 会 ▲ E ♥ ♥ ↑ Λ netogram 0 1 0 - 0 - 0 - 0 - 0 - 0 - 0 - 0 -	Calibration Curve Immode Types	Crigi		Weight		ISTD QC
npound Information : encorem 0 1 0 - 0.8- 0.7- 0.6- 0.5- 0.4- 0.5- 0.4- 0.5- 0.4- 0.5- 0.4- 0.5- 0.4- 0.5- 0.4- 0.1- 0.4- 0.5- 0.4- 0.5- 0.4- 0.5- 0.4- 0.5- 0.4- 0.5- 0.4- 0.5- 0.5- 0.4- 0.5-	Calibration Curve Image: Description of the state	Crigi		Weight		
npound Information :	Calibration Curve Image: Type: Image: T	Crig		Weight		15TO QC
npound Information :	 Calibration Curve (a) (b) (b) (b) (b) (b) (b) (b) (b) (b) (b	Origi		Weight		ISTD QC
npound Information :: ■ ● ● 八 玉 山 会 ▲ :	Calibration Curve Immediate	Crig		Weight:		ISTO QC
mpound Information : • ● ▲ 五 北 全 ▲ ! ● • • • • ▲ ▲ • ● ▲ 五 北 全 ▲ ! ● • • • • ● ▲ • ● ▲ 五 北 全 ▲ ! ● • • • • ● ▲ • ● ▲ 五 北 全 ▲ ! ● • • • • ● ▲ • ● ▲ 五 北 全 ▲ ! ● • • • • ● ▲ • ● ● ▲ 五 ● ● ▲ □ ● ▲ □ ● ● ▲ □ ● ● ● ● ● ● ● ● ●	Calibration Curve () () () () () () () () () ()	Oriot 60 40	-20 0	20	40 6	15TD QC

Figure 4 Default layout

Task 1. Set up a new batch

Steps	Detailed Instructions	Comments
	 b Select File > New Batch. The system opens the New Batch dialog box. c Navigate to the folder \ Your Directory \DrugsOfAbuse \. d Enter the batch filename iii_Test_01 and click Open. 	 If the default layout is not present, click Restore Default Layout on the toolbar before creating a new batch. Restore Default Layout
2 Add all the samples in the DrugsOfAbuse folder to the batch.	 a Select File > Add Samples: The system displays the Add Sample dialog box. b Click Select All to select all samples, and then 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 5 on the next page. 	 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.
	Add Samples Image: C:\QuantData\DrugsOfAbuse\ Batch Folder: C:\QuantData\DrugsOfAbuse\ CMAMBIk_01.d CMAMCal_L1.d CMAMCal_L2.d CMAMCal_L3.d CMAMCal_L5.d CMAMCal_L4.d CMAMOC_L2.d CMAMOC_L4.d CMAMGal_Sam_01.d CMAMSam_02.d CMAMSam_03.d Image: Copy Samples Select All OK Cancel	

Task 1. Set up a new batch

ilent MassHunter Quantitative Analysis - DrugsOfAbuse - iii Edit View Analyze Method Update Report Tools Hel Call View Analyze Batch ♥ : Layout: Tools Hel Call Call Call Call Call Call Call Call	Test_01 p ▲ Ø Restore Default Layout ♥ @	ISTD:	Time Segment:	<411>	
Compound: <	Test_01 p ↑ Restore <u>D</u> efault Layout ▼ ●	ISTD:	Time Segment:	<ali></ali>	
Edit View Analyze Method Update Report Iools Hel Image:	p [] [2] Restore Default Layout [] [2] [2] [2] [2] [2] [2] [2] [2] [2] [ISTD:	Time Segment:	<11>	<u> </u>
Image: Provide the second s	🔝 🗹 Restore Default Layout	ISTD:	Time Segment:	<11>	<u>∎</u> ≣₽₩₽₽₽₽₹
Ch Table Sample Type: <al> Compound: Sample Sample Image: Compound: Image: Compound:</al>	× @	ISTD:	Time Segment:	<ali></ali>	<u>■</u> ₩₩₩₩₩₩
Sample Sample Type: <all> Compound: Sample Sample Compound: Compound: <t< td=""><td>¥ (6)</td><td>ISTD:</td><td>Time Segment:</td><td><11></td><td><u> </u> </td></t<></all>	¥ (6)	ISTD:	Time Segment:	<11>	<u> </u>
Sample Sample V Name Type Level Acq. Date-Time Blank Blank 5/12/2006 1:43 Calib- Calib- Cal L 5/12/2006 1:54 Calib- Cal L 5/12/2006 1:54 Calib- Cal L 5/12/2006 2:03 Calib- Cal L 5/12/2006 2:03 Cal- Cal L 5/12/2006 2:03 QC-L CC L 5/12/2006 2:03 QC-L QC L 4/12/2006 2:03 Sample Sample Sample Sample					
Sample V Name Type Level Acq. Date-Time Blank Blan 5/12/2006 1:48 Calib- Calib- Cal 1.1 Calib- Cal 1.2 5/12/2006 1:54 Calib- Cal L.3 5/12/2006 1:57 Calib- Cal L.3 5/12/2006 2:03 Calib- Cal L.5 5/12/2006 2:03 Cal- Cal L.5 5/12/2006 2:03 QC-L QC L/2 5/12/2006 2:03 QC-L QC L/4 5/12/2006 2:03 Sample Sample Sample Sample					
♥ Name Type Level Acq. Date-Time Blank Blan 5/12/2006 1.48 Calib- Cal L1 5/12/2006 1.54 Calib- Cal L2 5/12/2006 1.54 Calib- Cal L3 5/12/2006 1.57 Calib- Cal L4 5/12/2006 2.03 Calib- Cal L5 5/12/2006 2.03 Calib- Cal L5 5/12/2006 2.03 Calib- Cal L5 5/12/2006 2.03 Qc-L QC L2 5/12/2006 2.06 Qc-L QC L4 5/12/2006 2.06 Qc-L QC L4 5/12/2006 2.02 Samp Samp Samp Samp					
Blank Blan 5/12/2006 1:48 Calib- Calib- L1 5/12/2006 1:51 Calib- Cal L2 5/12/2006 1:54 Calib- Cal L3 5/12/2006 1:57 Calib- Cal L3 5/12/2006 2:03 Calib- Cal L4 5/12/2006 2:03 Cal- Cal L5 5/12/2006 2:03 QC-L QC L2 5/12/2006 2:09 Samp Sam S/12/2006 2:12					
Calib- Cal L1 5/12/2006 1:51 Calib- Cal L2 5/12/2006 1:57 Calib- Cal L3 5/12/2006 1:57 Calib- Cal L3 5/12/2006 2:03 Calib- Cal L4 5/12/2006 2:03 Calib- Cal L5 5/12/2006 2:03 Qc-L QC L2 5/12/2006 2:06 Qc-L QC L4 5/12/2006 2:09 Samp Samp Samp Samp					
Calib- Cal L2 5/12/2006 1:54 Calib- Cal L3 5/12/2006 2:00 Calib- Cal L4 5/12/2006 2:00 Calib- Cal L5 5/12/2006 2:03 QC-L QC L2 5/12/2006 2:06 QC-L QC L4 5/12/2006 2:09 Samp Sam 5/12/2006 2:12					
Calib- Calib-<					
Calib Cal L5 9/12/2002.20 Calib Cal L5 9/12/2006.203 QC-L QC L2 5/12/2006.209 QC-L QC L4 5/12/2006.209 Samp Sam 5/12/2006.212					
QC-L QC L2 5/12/2006 2:06 QC-L QC L4 5/12/2006 2:09 Samp Sam 5/12/2006 2:12					
QC-L QC L4 5/12/2006 2:09 Samp Sam 5/12/2006 2:12					
Samp Sam 5/12/2006 2:12					
Samp Sam 5/12/2006 2:15					
Samp Sam 5/12/2006 2:18					
npound Information	×	Calibration Curve			
, ▋▣▣∧⊼业✿↓.☑↔≄Ѧ		A IND Type:	- Origin:	Weight:	
natogram		·			
ס 1					
0.9-		9 v10 2]			
0.8-	hand and and and and and	2 0.8-			
		ds 0.6-			
J. / -		8 0.0			
0.6-		0.4-			
0.5-		0.2-			
		0-			
J.4 -		-0.2-			
).3-		-0.4-			
0.2-		0.6			
		-0.0-			
J. 1		-0.8-			
		-1-	0 40 00 0		<u> </u>
U U.5 I I.5 Z Z.5 3 3.5 4 4.5 5 5.5 6 6	5.5 / 7.5 8 8.5 9 9.5 1	- 100 -80 -1	60 -40 -20 O	20 40	60 80 Concentratio
		U		1	

Figure 5 Batch table containing Drugs of Abuse samples before quantitation

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.

Steps		Detailed Instructions		(Comments	
1	Create a new method from acquired MRM data. • Use the calibration data file with the highest signal.	а	Use the mouse 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.	

📅 Agilent MassHunter Quantitative Analysis - Drug									
<u> </u>	<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				
🗄 🎦 🗁 🕞 🖾 🗍 💭 Analyze Batch 🛛 🥑 🕴 Layout:									
Batch Table									
🕴 Sample: 👔 📕 Sample Type: <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				
•		Calib-	Cal	L5	5/12/2006 2:03				
		QC-L	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				

b Select Method > Edit to switch to method editing mode.

The Method Edit Tasks appear in the column to the left of the View, as shown in Figure 6.

- Note that Figure 6 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.

Restore <u>D</u>efault Layout

Task 2. Set up a new method for the batch

Steps	Detailed Instructions Comments					
Agilent MassHunter Quantitative Anal	lysis - [New Method]					
Eile Edit View Analyze Method Up	idate <u>Report</u> Tools <u>H</u> elp					
Analyze Batch						
Method lasks X	Method Table					
New / Open Method	Time Segment: 🖛 <all> 🔹 🗰 Compound: 🔤 🔹 🖬 Reset Table View</all>					
Method Setup Tasks	Sample					
K MRM Compound Setup	Name Type Level Acq. Date-Time Data File					
K Retention Time Setup	▶ Calib-L5 Cal L5 5/12/2006 2:03 PM CMAMCal_L5.d					
😥 ISTD Setup						
Concentration Setup						
X Qualifier Setup						
Calibration Curve Setup						
🖉 Globals Setup	Sample Information					
Save / Exit	v + ↓ 1 % i Signal: <none> v</none>					
🞯 Validate +	TIC MRM (** -> **) CMAMCal_L5.d					
🖬 Save						
Save As	4-					
🔀 Exit	3-					
Manual Setup Tasks						
Outlier Setup Tasks	0-1 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					
Advanced Tasks						
G	x10 1					
	0.8-					
	0.6-					
	0.4-					
	0.2-					
	0					
	0 Compounds (0 total) 0					

Figure 6 Method Edit mode

Task 2. Set up a new method for the batch

Steps	Detailed Instructions	Comments		
	 c Under Method Tasks in the sidebar to the left of the View, select New/Open Method > New Method from Acquired MRM Data. The system displays a <i>Please select a sample folder</i> dialog box. d Select CMAMCal_L5.d and click Open to import acquisition method information 	 You can also select Method > New > New Method from Acquired MRM Data. The figure below shows the default layout for the level 5 calibration standard. 		

File Edit Year Analyze Method Layout Method Tasks * Concentration Concentration Concentration New Method from Acquired MRM Da. Name Type Level Acq DateTime New Method from Acquired MRM Da. Name Type Level Acq DateTime New Method from Acquired Scan Dat. Name Type Level Acq DateTime Open Method from Existing Batch Name Ts Transition Scan Method Setup Calibration Setup 136.2 > 91.4 MRM Target Concentration Setup Calibration Curve Setup 136.2 > 119.4 136.2 > 119.4 26.6 Concentration Setup Calibration Curve Setup Name Ts Transition Scan Collaritier Name Ts Transition Scan Target Qualifier Name Ts Transition Scan Target Calibration Curve Setup Name Ts Transition Scan Colaritier Ouantifier Ouantifier Ouantifier Stave As Coccaine 1304.1 > 132.0 MRM Target Method Setup Tasks Name Ts Transition Scan Method Setup Tasks Name Ts Transition Scan Method Setup Coccaine 1304.1 > 132.0 MRM Target Method Setup Tasks Name Ts Transition Scan Method Setup Tasks Name Ts	🖥 Agilent MassHunter Quantitative Analysis - [New Method]								
Image: Solution of the set of the	<u>Elle Edit View Analyze Method Update Report Tools H</u> elp								
Method Tasks Method Table × New / Open Method Ime Segment: < Compound: EgetTable View New Method from Acquired Scan Det New Method from Existing Batch Name Type Level Acq. Date-Time A	🗅 🗁 📕 📭 l 🗊 Analyze Batch 🛛 🥥 🚦 Layout: 🔜 🔢 🔛 🔛 🏧 🏧 🖉 Restore Default Layout								
New / Open Method Time Segment: * <all> Compound: * Respect Table View New Method from Acquired MRM Da. New Method from Acquired Scan Dat. New Method from Existing File Open Method from Existing Blach Name Type Level Acq. Date-Time CMAM Open Method from Existing File Open Method from Existing Blach Name TS Transition Scan MRM Compound Setup Precursor Ion Product Ion Transition Rel. Resp. U Calibration Curve Setup Globals Setup Mame TS Transition Scan Scan Globals Setup Save As Exit Name TS Transition Rel. Resp. U Validate Qualifier Precursor Ion Product Ion Transition Scan Scan Save As Exit Mame TS Transition Scan Scan<th colspan="9">Method Tasks x Method Table x</th></all>	Method Tasks x Method Table x								
P New Method from Acquired MRM Da. New Method from Acquired Scan Dat. New Method from Acquired Scan Dat. New Method from Existing File Name Type Level Acq Date-Time Open Method from Existing File Outantifier CMAMCal_L5.d CMAMC Open Method from Existing Blach Name TS Transition Scan Method Setup Tasks Amp 1 136.2 -> 91.4 MRM Target Method Setup Tasks Amp 1 136.2 -> 91.4 MRM Target Qualifier Qualifier Qualifier U 136.2 119.4 26.5 Qualifier Setup Globals Setup Name TS Transition Scan ISTD Globals Setup Qualifier Qualifie	New / Open Method								
New Method from Acquired Scan Dat. New Method using Manual Setup Name Type Level Acq. Date-Time We Method using Manual Setup CMAMCal_L5.d CMAMCal_L5.d CMAM Open Method from Existing File Open Method Setup Tasks Mame TS Transition Scan Method Setup Tasks Amp 1 136 2-> 91.4 MRM Target MRM Compound Setup Amp 1 136 2-> 91.4 MRM Target Califier Qualifier Qualifier U Transition Rel. Resp. U Store Jostup 36.2 1 141.1 > 93.4 MRM ISTD Globals Setup Qualifier Validate U 141.1 124.4 141.1 > 124.4 26.4 Qualifier Validate Validate Validate U 141.1 124.4 141.1 > 124.4 26.4 Quantifier Validate Validate <t< th=""><th>New Method from Acquired MRM Da</th><th>Sample</th><th></th><th></th><th></th><th>^</th></t<>	New Method from Acquired MRM Da	Sample				^			
New Method using Manual Setup Image: Constraint of Constraints of Constand constand constraints of Constraints of Constraints	New Method from Acquired Scan Dat	Name	Туре	Level	Acq. Date-Time				
Open Method from Existing File Open Method from Existing Batch Method Setup Tasks (MRM Compound Stup C MRM Compound Stup (Retention Time Setup 2 ISTD Setup 2 Concentration Setup (Calibration Curve Setup (Calibration Curve Setup (Globals Setup Save / Exit (Save As 3 Exit Manual Setup Tasks (New Condifier (New Condifier (New Condifier (New Condifier (Delete (New Condifier (Delete	New Method using Manual Setup	CMAMCal_L5.d				CMAM			
Name TS Transition Scan Method Setup Tasks Amp 1 136.2 -> 91.4 MRM Target MRM Compound Setup Qualifier Qualifier Qualifier U Y Store Store 136.2 -> 91.4 MRM Target Qualifier Qualifier U 136.2 -> 91.4 MRM Target Qualifier Setup 136.2 -> 119.4 136.2 -> 119.4 26.5 Quantifier Qualifier Setup Mame TS Transition Scan ISTD Qualifier Setup Amp-d5 1 141.1 -> 93.4 MRM ISTD Qualifier	Øpen Method from Existing File	Quantifier							
Method Setup Tasks Amp 1 136.2 > 91.4 MRM Target MRM Compound Setup Qualifier Precursor Ion Product Ion Transition Rel. Resp. U StD Setup 136.2 119.4 136.2 > 91.4 MRM Target Qualifier Setup 136.2 119.4 136.2 > 91.4 MRM Less Qualifier Setup Calibration Curve Setup 136.2 119.4 136.2 > 91.4 MRM IstD Globals Setup Name TS Transition Scan ISTD Globals Setup Amp-d5 1 141.1 > 93.4 MRM ISTD Quantifier Oualifier Precursor Ion Product Ion Transition Rel. Resp. U Validate Save Save Name TS Transition Scan Scan Save As Cocaine 1 304.1 > 182.0 MRM Target Quantifier Qualifier Qualifier Qualifier Qualifier Qualifier New Collibration Level New Collibration Level Name TS Transition Scan	Open Method from Existing Batch	Name	TS	Transition	Scan				
MRM Compound Setup Qualifier Retention Time Setup Precursor Ion Product Ion Transition Rel. Resp. U ISTD Setup I36.2 119.4 136.2-> 119.4 26.5 Quantifier I36.2 119.4 136.2-> 119.4 26.5 Quantifier Mame TS Transition Scan Globals Setup Amp-d5 1 141.1->93.4 MRM ISTD Save / Exit Precursor Ion Product Ion Transition Rel. Resp. U Validate 141.1 124.4 141.1->124.4 26.4 Quantifier Ouantifier Save Save Save Save Save Save Save Scan Image: I	Method Setup Tasks	C Amp	1	136.2 -> 91.4	MRM	Target			
Retention Time Setup Precursor Ion Product Ion Transition Rel. Resp. U ISTD Setup 136.2 119.4 136.2 119.4 26.5 Concentration Setup Quantifier Quantifier Quantifier Quantifier Globals Setup Amp-d5 1 141.1 > 93.4 MRM ISTD Save / Exit Precursor Ion Product Ion Transition Rel. Resp. U Validate 141.1 124.4 141.1 > 124.4 26.4 Quantifier Quantifier Quantifier Quantifier Save As Cocaine 1 304.1 > 182.0 MRM Target Quantifier Quantifier<	K MRM Compound Setup	Qualifier							
2 ISTD Setup 136.2 119.4 136.2.> 119.4 26.5 2 Concentration Setup Name TS Transition Scan Scan 2 Calibration Curve Setup Name TS Transition Scan Scan 3 Globals Setup Qualifier U Validate U Validate U Validate Validate U Validate Cocaine 1 141.1 > 124.4 141.1 > 26.4 Qualifier 3 Save As Save As Cocaine Transition Scan	K Retention Time Setup	Precursor Ion	Product Ion	Transition	Rel. Resp.	U			
Concentration Setup Quantifier Qualifier Setup Amp-d5 Globals Setup Int1.1>93.4 Globals Setup Qualifier Save / Exit Precursor Ion Validate 141.1>124.4 Save As 141.1 Save As Transition Save As Name Exit Name Manual Setup Tasks Oucalifier New Califier Quantifier New Califier Quantifier New Califier Quantifier New Califier Quantifier Quantifier 04.1 Rev Califier 03.1 New Califier Quantifier Quantifier 04.1 Quantifier 30.1 New Califier Quantifier Quantifier Quantifier Quantifier Quantifier Quantifier Quantifier New Califier Quantifier Quantifier Quantifier Quantifier Quantifier Quantifier Quantifier Quantifier Quant	😥 ISTD Setup	136.2	119.4	136.2 -> 119.4	26.5	,			
Name TS Transition Scan Calibration Curve Setup Amp-d5 1 141.1 >> 93.4 MRM ISTD Globals Setup Qualifier U U 141.1 >> 93.4 MRM ISTD Save / Exit Precursor Ion Product Ion Transition Rel. Resp. U Validate 141.1 124.4 141.1 >> 124.4 26.4 Quantifier Save As Name TS Transition Scan Image: Coccaine 1 304.1 >> 182.0 MRM Target Qualifier Qualifier U 20.4.1 82.0 3.8 Image: Coccaine 1 30.4.1 >> 82.0 3.8 Image: Coccaine Image: Cocc	🚀 Concentration Setup	Quantifier	Quantifier						
Calibration Curve Setup Amp-d5 1 141.1 -> 93.4 MRM ISTD Globals Setup Qualifier Precursor Ion Product Ion Transition Rel. Resp. U Validate 141.1 124.4 141.1 -> 124.4 26.4 Quantifier Save Save Save Name TS Transition Scan ISTD Save As Name TS Transition Scan ISTD Lexit Qualifier Qualifier Qualifier Isto Isto Isto Manual Setup Tasks Precursor Ion Product Ion Transition Rel. Resp. U New Compound 304.1 82.0 304.1 -> 82.0 3.8 Quantifier New Calibration Level Name TS Transition Scan ISTD Outlier Setup Tasks Qualifier Qualifier Isto MRM ISTD Qualifier Name TS Transition Scan ISTD Qualifier Qualifier Qualifier Isto MRM ISTD Qualifier	🛣 Qualifier Setup	Name	TS	Transition	Scan				
Globals Setup Qualifier Save / Exit Precursor Ion Product Ion Transition Rel. Resp. U Validate 141.1 124.4 141.1 -> 124.4 26.4 Save As Quantifier Cocaine 1 304.1 -> 182.0 MRM Target Manual Setup Tasks Qualifier U Qualifier U 304.1 -> 82.0 3.8 New Compound New Calibration Level Name TS Transition Rel. Resp. U New Calibration Level Name TS Transition Rel. Resp. U Quantifier Oucartifier Oucartifier Name TS Transition Rel. Resp. U New Calibration Level Name TS Transition Scan Scan <th>🛠 Calibration Curve Setup</th> <th>a Amp-d5</th> <th>1</th> <th>141.1 -> 93.4</th> <th>MRM</th> <th>ISTD</th>	🛠 Calibration Curve Setup	a Amp-d5	1	141.1 -> 93.4	MRM	ISTD			
Save / Exit Precursor Ion Product Ion Transition Rel. Resp. U Validate 141.1 124.4 141.1 > 124.4 26.4 Save Save Save As Name TS Transition Scan Image: Constraint of the second of	🕈 Globals Setup	Qualifier							
Validate 141.1 124.4 141.1 > 124.4 26.4 Save Save As Name TS Transition Scan Strit Name TS Transition Scan Image: Coccaine 1 304.1 >> 182.0 MRM Target Qualifier Qualifier Image: Coccaine 1 304.1 >> 182.0 MRM Target New Compound New Qualifier Image: Coccaine -0.3 Image: Cocccaine -0.3 Image: Coccaine -0.	Save / Exit	Precursor Ion	Product Ion	Transition	Rel. Resp.	U			
Save Save As Save As Name Transition Scan Exit Cocaine Manual Setup Tasks Ualifier New Compound Outlifier New Calibration Level Outlifier New Calibration Level Name Delete Cocaine-d3 Outlifier Transition Rest Cocaine-d3 Qualifier Stransition Qualifier Outlifier Qualifier Stransition Qualifier Stransition Qualifier Outlifier Qualifier Stransition Qualifier Name Transition Scan Qualifier Outlifier Qualifier Qualifier Qualifier Qualifier Qualifier Qualifier	谢 Validate	141.1	124.4	141.1 -> 124.4	26.4	,			
Save As Name TS Transition Scan 3 Exit Coceine 1 304.1 -> 182.0 MRM Target Qualifier Qualifier Qualifier Qualifier Qualifier Qualifier New Compound New Collibration Level Quantifier Quantifier Quantifier New Collibration Level Name TS Transition Scan Quantifier Quantifier Quantifier Scan Scan Quantifier Quantifier Quantifier Scan Scan Qualifier Qualifier Name TS Transition Scan Qualifier Qualifier Qualifier Name TS Transition Scan Qualifier Qualifier Qualifier Name TS Transition Scan	Save	Quantifier							
Sexit Coceine 1 304.1 -> 182.0 MRM Target Manual Setup Tasks Qualifier Qualifier U 304.1 -> 82.0 3.8 New Compound New Collibration Level Quantifier U 3.8 3.8 New Calibration Level Name TS Transition Scan Outlier Setup Tasks Qualifier Qualifier Qualifier Advanced Tasks Manuel Tasks Name TS Transition	Save As	Name	TS	Transition	Scan				
Manual Setup Tasks Qualifier Precursor Ion Product Ion Transition Rel. Resp. U New Qualifier 304.1 82.0 304.1 > 82.0 3.8 New Collibration Level Quantifier Image: Coccaine-d3 1 307.1 > 185.0 MRM Outlier Setup Tasks Qualifier Qualifier Image: Coccaine-d3 1 307.1 > 185.0 MRM ISTD Advanced Tasks Image: Coccaine-d3 Image: Coccaine-d3 <t< th=""><th>X Exit</th><th>Cocaine</th><th>1</th><th>304.1 -> 182.0</th><th>MRM</th><th>Target</th></t<>	X Exit	Cocaine	1	304.1 -> 182.0	MRM	Target			
Precursor Ion Product Ion Transition Rel. Resp. U R New Collibration Level 304.1 82.0 304.1 >> 82.0 3.8 New Collibration Level Quantifier Image: Collibration Collibra	Manual Setup Tasks	Qualifier							
New Composition 304.1 82.0 304.1 -> 82.0 3.8 New Qualifier Quantifier Quantifier Name TS Transition Scan New Collibration Level Name TS Transition Scan Coccaine-d3 1 307.1 -> 185.0 MRM ISTD Outlier Setup Tasks Qualifier Qualifier Vertice Vertice <th>A New Concerned</th> <th>Precursor Ion</th> <th>Product Ion</th> <th>Transition</th> <th>Rel. Resp.</th> <th>U</th>	A New Concerned	Precursor Ion	Product Ion	Transition	Rel. Resp.	U			
New Calibration Level Quantifier New Calibration Level Name TS Transition Scan Outlier Setup Tasks Advanced Tasks	7 New Compound	304.1	82.0	304.1 -> 82.0	3.8	i i i i i i i i i i i i i i i i i i i			
Name TS Transition Scan Outlier Setup Tasks Coceine-d3 1 307.1 -> 185.0 MRM ISTD Qualifier Qualifier Vertice Vertice Vertice Vertice	New Calibration Level	Quantifier			1				
Outlier Setup Tasks Cocaine-d3 1 307.1-> 185.0 MRM ISTD Qualifier	X Delete	Name	TS	Transition	Scan				
Advanced Tasks	Outlier Setup Tacks	Cocaine-d3 1 307.1 -> 185.0 MRM							
Advanced Tasks		Qualifier			1	~			
	Advanced Tasks	<				>			
4 Compounds (4 total) 4 ISTD (4 total)				4 Cc	mpounds (4 total) 4 ISTD	4 total) .::			

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.

Steps		De	Detailed Instructions		Comments	
1	Check the new quantitation method created from the imported acquisition method for MRM transitions.	a	Under Method Tasks in the sidebar to the left of the View, 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 A	Analysis	- [New Method]						
Eile Edit View Analyze Method	Update	<u>Report T</u> ools <u>H</u> elp						
🗄 🐚 🕞 🖌 🖓 🖓 🖓 🖓 🖓 🖓	0 ! L	ayout: 🔜 😥 🖾 🔟 🖊	Restore Default	Layout				
Method Tasks ×	Meth	od Table						×
New / Open Method	Tim	e Segment: 👄 <all></all>	👻 📫 📔 Comp	ound: 🐖	💌 📑 🛛 <u>R</u> eset T	able View		
New Method from Acquired M	Sa	mple						
New Method from Acquired Sc		Name	Туре	Level	Acq. Date-Time	Data File		
New Method using Manual Set		CMAMCal_L5.d				CMAMCal_L5.d		
Øpen Method from Existing Fil		Quantifier						1
Open Method from Existing Ba		Name	TS	Transition	Scan	Туре	Precursor Ion	Product Ion
Method Setup Tasks		Amp	1	136.2 -> 91.4	MRM	Target	136.2	91.4
		Amp-d5	1	141.1 -> 93.4	MRM	ISTD	141.1	93.4
K MRM Compound Setup	-	Cocaine	1	304.1 -> 182.0	MRM	Target	304.1	182.0
K Retention Time Setup	-	Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD	307.1	185.0
🖈 ISTD Setun		MDMA	1	194.2 -> 163.2	MRM	Target	194.2	163.2
to o o o o	4	MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	199.2	164.3
-7 Concentration Setup	-	Meth	1	150.1 -> 119.3	MRM	Target	150.1	119.3
🕂 Qualifier Setup	, Lin	Meth-d5	1	155.1 -> 92.3	MRM	ISTD	155.1	92.3

Task 3. Set up target compounds

Detailed Instructi	ons			Comr	nents				
 b To inspect the data, click Met Retention Time 	imported re hod Setup e Setup.	eter Tas	ntion tim : ks >	e • You for	u can mo individua	dify al co	data fiel mpounc	ds in blu Is.	ıe
Nglient MassHunter Quantitative Ble Edit View Analyze Method	Analysis - [New Meth Update Report To	odj ools <u>H</u>	elp 副 (石) (忍) - Rester	re Default Lavout					
Mothod Tasks	Method Table	3 633 6	TO REALIZED COMPANY	e geraar cajour					
Mitting Tunio	HILLING FUCK		2012/2014			-			
New / Open Method	Time Segment:	<al></al>	1 - C	Compound:	50 J	el Bese	t Table View		
P New Method from Acquired_	Sample								
New Method from Acquired_	Name	Type	Level	Acq Date-Time	Data File				
New Method using Manual	► CMAMCal_L5			1	CMAMCal_L5 d				
Cone Method from Existing	Ounntifier								
Open Method from Existing	Mana	TO	Transfer	Page	Turn	OT	Left DT Delle	Diale DT Dalla	DT Daha U
open menos non externing.	Iters	15	I rensition	Scan	туре	7.101	Left RT Detta	Right RT Delta	RI Deta U
Method Setup Tasks	Amp	1	141 1 -> 91.4	MPM	Isto	2.076	1.000	1.000	Minutes
K MRM Compound Setup	Cocaine	1	304 1 -> 182 0	MRM	Target	2.448	1.000	1.000	Minutes
/C Retention Time Setup	Cocaine-d	1	307.1 -> 185.0	MRM	ISTD	2.448	1.000	1.000	Minutes
1STD Setup	MDMA	1	194.2 -> 163.2	MRM	Target	2.271	1.000	1.000	Minutes
2 Concentration Setup	MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	2.268	1.000	1.000	Minutes
2 Contentibilition Setup	Meth	1	150.1 -> 119.3	MRM	Target	2.237	1.000	1.000	Minutes
Za Qualmer Setup	[wteth-do	1	100.1-2.95.3	1005100	1310	2.231	1.000	1.000	namutes.

2 Set up ISTD compounds.

-

- Assign the corresponding deuterated compound as the internal standard (ISTD) for each target compound.
- a Select 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.

ng Agilent MassHunter Quantitativ	/e A	nalysi	s - [New Meth	od]				
<u>File E</u> dit <u>V</u> iew <u>A</u> nalyze <u>M</u> etho	bd	<u>U</u> pdat	e <u>R</u> eport <u>T</u> e	ools <u>H</u>	elp			
🏠 🗁 🔛 🖬 🖓 🖓 Analyze Batcl		0 : 1	Layout: 📆 🛛		🛾 🔼 🕺 Restore	e <u>D</u> efault Layout		
Method Tasks ×	N	letho	d Table					
New / Open Method	:	Time	Segment: ┿	<all></all>	▼ ⇒	Compound: 🔙 C	Cocaine 💌	💼 🛛 <u>R</u> eset Table View
P New Method from Acquired		Sam	ple					
New Method from Acquired			Name	Туре	Level	Acq. Date-Time	Data File	
New Method using Manual		C	MAMCal_L5				CMAMCal_L5.d]
🕒 Open Method from Existing		Q	luantifier					
Open Method from Existing			Name	TS	Transition	Scan	Туре	ISTD Compound Name
Method Setup Tasks			Amp	1	136.2 -> 91.4	MRM	Target	Amp-d5
			Amp-d5	1	141.1 -> 93.4	MRM	ISTD	<none></none>
MRM Compound Setup		•	Cocaine	1	304.1 -> 182.0	MRM	Target	Cocaine-d3
K Retention Time Setup		-	Cocaine-d	1	307.1 -> 185.0	MRM	ISTD	Amp-d5
क्ती ISTD Setup			MDMA	1	194.2 -> 163.2	MRM	Target	Cocaine-d3
Concentration Setup			MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	MUMA-05
- concentration Setup		-	Meth	1	150.1 -> 119.3	MRM	Target	chanas
🛣 Qualifier Setup			Meth-d5	1	155.1 -> 92.3	MRM	ISTD	<nou65< td=""></nou65<>

Task 3. Set up target compounds

eps		I	Detailed Instru	ctions	Comr	nents		
		(Select the IS the target co Enter the IST for each ISTI 	TD name asso mpound. D Conc (Conc D compound.	ciated with entration)			
Method Table								
Time Segment: 🦛 <a< td=""><td>.ll></td><td>🝷 🔿 📔 Comp</td><td>ound: 🔙 Meth-d5</td><td>▼ ● <u>R</u>ese</td><td>et Table View</td><td></td><td></td><td></td></a<>	.ll>	🝷 🔿 📔 Comp	ound: 🔙 Meth-d5	▼ ● <u>R</u> ese	et Table View			
Sample								
Name	Туре	Level	Acq. Date-Time	Data File				
CMAMCal_L5.d				CMAMCal_L5.d				
Quantifier								
Name	TS	Transition	Scan	Туре	ISTD Compound Name	ISTD Flag	ISTD Conc.	Time Re
Amp	1	136.2 -> 91.4	MRM	Target	<none></none>			
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	<none></none>		50.0000	
Cocaine	1	304.1 -> 182.0	MRM	Target	<none></none>			
Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD	<none></none>	V	50.0000	
MDMA	1	194.2 -> 163.2	MRM	Target	<none></none>			
MDMA-d5	1	199.2 -> 164.3	MRM	ISTD	<none></none>	~	50.0000	
Meth	1	150.1 -> 119.3	MRM	Target	<none></none>			
Meth-d5	1	155.1 -> 92.3	MRM	ISTD	<none></none>		50.0000	

Task 4. Set up quantitation

Task 4. Set up quantitation

This task presents instructions for setting up the quantitation parameters for the method.

- Calibration levels
- Qualifier ions
- Calibration curve fit

Step	S	De	etailed Instructions	Comments
1 (Create five calibration levels for ach compound. Set the highest concentration for amphetamine of 125. Set a Dilution Pattern of 1:5:2:2.5:2 for amphetamine. Compare the concentrations for the five levels with the Dilution Pattern	a b c	Select Method Setup Tasks > Concentration Setup, and type 125 in the Dil. High Conc. column for amphetamine (Amp). Type 1:5:2:2.5:2 in the Dil. Pattern column for Amp. Make sure Level Name Prefix is L and # of Levels is 5 in the Serial Dilution toolbar	

📅 Agilent MassHunter Quantitat	ive A	nalys	is - [New Meth	od]							×
<u> </u>	hod	Upda	te <u>R</u> eport <u>T</u> o	ools <u>H</u> elp							
🗄 🛅 🗁 🛃 🖬 🕯 💭 Analyze Bat	tch]	0 :	Layout: 🔙 🛛		Kestore Defa	ult Layout					
Method Tasks ×	Me	ethoo	i Table							;	×
New / Open Method	÷т	ime S	Segment: 🦛 <	All>	🔫 🔿 🕴 Compo	und: 💓 Amp	- 🖬	<u>R</u> eset Table View			
Method Setup Tasks	: L	evel I	Name Prefix: L	e.	# of Levels: 5	<u>C</u> rea	te Levels				
/ MRM Compound Setup	1	Samp	ble								
Retention Time Setup			Name	Туре	Level	Acq. Date-Time	Data File				
😥 ISTD Setup		C	MAMCal_L5.d				CMAMCal_L	1			
🦃 Concentration Setup		Q	uantifier								1
🕂 Qualifier Setup			Name	TS	Transition	Scan	Туре	Dil. High Conc.	Dil. Pattern	Units	1
👯 Calibration Curve Setup		•	Amp	1	136.2 -> 91.4	MRM	Target	125.0000	1:5:2:2.5:2	✓ ng/ml	
Clobals Setup		-	Amp-d5	1	141.1 -> 93.4	MRM	ISTD			nq/ml	
			Cocaine	1	304.1 -> 182.0	MRM	Target			ng/ml	
Save / Exit			Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD			ng/ml	
🕅 Validato			MDMA	1	194.2 -> 163.2	MRM	Target			ng/ml	
Vandate			MDMA-d5	1	199.2 -> 164.3	MRM	ISTD			ng/ml	_
Bave Save		-	Meth	1	150.1 -> 119.3	MRM	Target			ng/ml	_
Savo Ac		-	Meth-d5	1	155.1 -> 92.3	MRM	ISTD			ng/ml	

Figure 7 Creating five calibration levels for first compound

Task 4. Set up quantitation

Steps			D	etailed	Instruction	s		Comments		
			d e	Click C Compa levels Conce	Create Leve are the new with Dilutio ntration and	ls . ly created ca n High d Dilution Pa	libration ttern.	 After you table for a program t other targ 	create the ca amphetamine to copy this ta get compound	libration , you tell the ble to the Is in step 2.
Bagilent MassHunter Quantit	ative A	nalv	sis - [New Metho	41				•		
File Edit View Analvze M	ethod	Upd	ate Report Too	ls Help						لك تك
👔 🗁 📕 İ 🖬 İ 💭 Analyze B		0	Layout: 🔜 🔛		🕺 Restore <u>D</u> efa	ult Layout				
Method Tasks	< M	etho	d Table							
New / Open Method	1	Time	Segment: 🦛 <a< td=""><td> ></td><td>🕶 📫 🗌 Compo</td><td>ound: 🔙 Amp</td><td>-</td><td><u>R</u>eset Table View</td><td></td><td></td></a<>	>	🕶 📫 🗌 Compo	ound: 🔙 Amp	-	<u>R</u> eset Table View		
Method Setup Tasks	÷ L	.evel	Name Prefix: L		# of Levels: 5	<u>C</u> reat	te Levels			
MBM Compound Setup	-	C	Quantifier							
Retention Time Setup			Name	TS	Transition	Scan	Туре	Dil. High Conc.	Dil. Pattern 🗸	Units
😥 ISTD Setup		•	Amp	1	136.2 -> 91.4	MRM	Tarqet	125.0000	1:5:2:2.5:2	ng/ml
🪀 Concentration Setup			Calibration							
🕂 Qualifier Setup			Level	Conc.						
🚀 Calibration Curve Setup			L1	2.5000						
Globals Setup			L2	5.0000						
Save / Exit			L3	25.000						
	-		1.5	125.00						

2 Copy the calibration levels and concentrations to the other compounds.

name e a la composition de la

- Close the Compound Information window.
- · Compare the calibration setup for the four compounds.
- a Select Method > Copy Calibration Levels To...

The system displays the Copy Calibration Levels dialog box.

b Click **Select All**, and then click **OK**.

	15	RT	Transition	ISTD Flag	
Cocaine	1	2.448	304.1 -> 182.0		
IDMÀ	1	2.271	194.2 -> 163.2		
leth	1	2.237	150.1 -> 119.3		

Steps	Detailed Instructions	Comments	
	 c Close the Compound Inforwindow and the Sample Inwindow in the lower half oQuantitative Data Analysis d Browse the Method Tablethe calibration concentratianong the four target comAmp, Cocaine, Meth and Name 	mation formation of the s main view. to compare on setup upounds, ADMA	

Namo	TS	Transition	Scan	Type	Dil High Conc	Dil Pattorn /	11
Name	15	Transition	Scan	Type	Dil. High Conc.	Dii. Pattern	U
Amp	1	136.2 -> 91.4	MRM	larget	125.0000	1:5:2:2:5:2	ng/m
Calibration							
Level	Conc.						
L1	2.5000						
L2	5.0000						
L3	12.500						
L4	25.000						
L5	125.00						
luantifier							
Name	TS	Transition	Scan	Туре	Dil. High Conc.	Dil. Pattern	U
Amp-d5	1	141.1 -> 93.4	MRM	ISTD	-		na/m
Cocaine	1	304.1 -> 182.0	MRM	Target	125 0000	1.5.2.2.5.2	na/m
Level L1 L2	Conc. 2.5000 5.0000						
Level L1 L2 L3 L4 L4	Conc. 2.5000 5.0000 12.500 25.000						
Level	Conc. 2.5000 5.0000 12.500 25.000 125.00						
Level L1 L2 L3 L4 L5 tuantifier	Conc. 2.5000 5.0000 12.500 25.000 125.00						4
Level L1 L2 L3 L4 L5 wantifier Name	Conc. 2.5000 5.0000 12.500 25.000 125.000 125.00	Transition	Scan	Туре	Dil. High Conc.	Dil. Pattern 1	U
Level L1 L2 L3 L4 L5 uantifier Name Cocaine-d3	Conc. 2.5000 5.0000 25.000 25.000 125.00 125.00 TS	Transition 307.1 -> 185.0	Scan MRM	Type	Dil. High Conc.	Dil. Pattern /	U
Level L1 L2 L3 L4 L5 uantifier Name Cocaine-d3 MDMA	Conc. 2.5000 5.0000 12.500 25.000 125.00 TS TS 1 1	Transition 307.1 -> 185.0 194.2 -> 163.2	Scan MRM MRM	Type ISTD Target	Dil. High Conc.	Dil. Pattem /	U ng/m
Level L1 L2 L3 L4 L5 contifier Name Cocaine-d3 MDMA Calibration	Conc. 2.5000 5.0000 12.500 25.000 125.00 125.00 TS TS 1 1	Transition 307.1 -> 185.0 194.2 -> 163.2	Scan MRM MRM	Type ISTD Target	Dil. High Conc.	Dil. Pattern /	U ng/m
Level L1 L2 L3 L4 L5 tuantifier Name Cocaine-d3 MDMA Coalibration Level	Conc. 2.5000 5.0000 12.500 25.000 125.00 TS 1 1 Conc.	Transition 307.1 -> 185.0 194.2 -> 163.2	Scan MRM MRM	Type ISTD Target	Dil. High Conc.	Dil. Pattern /	U ng/m ng/m
Level L1 L2 L3 L4 L5 Name Cocaine-d3 MDMA Calibration Level	Conc. 2.5000 5.0000 12.500 25.000 125.00 TS 1 1 1 Conc. 2.5000	Transition 307.1 -> 185.0 194.2 -> 163.2	Scan MRM MRM	Type ISTD Target	Dil. High Conc.	Dil. Pattem /	U ng/m ng/m
Level L1 L2 L3 L4 L5 tuantifier Name Cocaine-d3 MDMA Calibration Level L1 L2	Conc. 2.5000 5.0000 12.500 125.000 125.000 TS 1 1 1 Conc. 2.5000 5.0000	Transition 307.1 -> 185.0 194.2 -> 163.2	Scan MRM MRM	Type ISTD Target	Dil. High Conc. 125.0000	Dil. Pattem /	U ng/m
Level L1 L2 L3 L4 L5 tuantifier Name Cocaine-d3 MDMA Colibration Level L1 L2 L3 L3 L4 L4 L5 L4 L5 L5 L5 L5 L5 L5 L5 L5 L5 L5	Conc. 2.5000 5.0000 12.500 12.500 125.00 125.00 TS 1 1 Conc. 2.5000 5.0000 12.500	Transition 307.1 -> 185.0 194.2 -> 163.2	Scan MRM MRM	Type ISTD Target	Dil. High Conc.	Dil. Pattern /	U Ing/m
Level L1 L2 L3 L4 L5 twantifier Name Cocaine-d3 MDMA Calibration Level L1 L2 L3 L4 L4 L5 L5 L5 L5 L5 L5 L5 L5 L5 L5	Conc. 2 5000 5 0000 12 500 125 000 125 00 TS TS 1 1 1 Conc. 2 5000 5 0000 12 500 2 5000 5 0000 12 500 2 5000 12 500 5 0000 12 500 5 0000 12 500 5 0000 12 500 12 500 5 0000 12 500 12 500	Transition 307.1 -> 185.0 194.2 -> 163.2	Scan MRM MRM	Type ISTD Target	Dil. High Conc.	Dil. Pattem /	U nq/m

St	eps	Detailed Instructions	Comments			
3	 Set up qualifier ions and a calibration curve. Review the Qualifier setup parameters. Change the default curve origin from Linear to Force. 	a Select Method Tasks > 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. 			

ng Agrient Massnunter Quantitat	tive Anal	ysis - [New Method]]						
<u>F</u> ile <u>E</u> dit <u>V</u> iew <u>A</u> nalyze <u>M</u> et	hod <u>U</u> p	date <u>R</u> eport <u>T</u> ools	<u>H</u> elp						
🗋 🗁 📓 🖬 🕻 🗊 Analyze Bal	tch 🛛 🕜	Layout: 🔜 🔝		Restore Default La	yout				
Method Tasks ×	Meth	od Table							
New / Open Method	Tim	e Segment: 🦇 <all:< th=""><th>> •</th><th>🗢 🗎 Compound: </th><th>Meth</th><th>🕶 📑 🛛 <u>R</u>eset Tab</th><th>le View</th><th></th><th></th></all:<>	> •	🗢 🗎 Compound:	Meth	🕶 📑 🛛 <u>R</u> eset Tab	le View		
Method Setup Tasks	Leve	el Name Prefix: L	#	of Levels: 5	<u>C</u> reate Level	ls			
K MRM Compound Setup	Sar	mple							
K Retention Time Setup		Name	Туре	Level	Acq. Date-Time	Data File			
😥 ISTD Setup		CMAMCal_L5.d	•	1		CMAMCal_L5.d			
🪀 Concentration Setup		Quantifier			1	-			
🕂 Qualifier Setup		Name	TS	Transition	Scan	Туре	Precursor Ion	Product Ion	Uncertainty
Calibration Curve Setup	8	Amp		1 136.2 -> 91.4	MRM	Target	136.2	91.4	Relative
Globals Setup		Qualifier							
Save / Exit		Precursor Ion	Product Ion	Transition	Rel. Resp.	Uncertainty	Area Sum		
🕅 Validate		136.2	119	4 136.2 -> 119.4	26.5	20.0			
En Covo		Quantifier							
Save As		Name	TS	Transition	Scan	Туре	Precursor Ion	Product Ion	Uncertainty
		Amp-d5		1 141.1 -> 93.4	MRM	ISTD	141.1	93.4	Relative
Exit		Qualifier					1		
Manual Setup Tasks		Precursor Ion	Product Ion	Transition	Rel Resp	Uncertainty	Area Sum		
Outlier Setup Tasks		141.1	124	4 141.1 -> 124.4	26.4	20.0			
Advanced Tasks		Quantifier							
	-	Name	TS	Transition	Scan	Type	Precursor Ion	Product Ion	Uncertainty
		Cocaine	10	1 304 1 -> 182 0	MRM	Target	304.1	182.0	Relative
		Qualifier			1				
		Deseurseles	Denduction	Transition	Del Dese	Unandalate	Arra Curra		
		Precursor ion	Product Ion		Rei. Resp.	Oncertainty	Area Sum		
		0.04.1	02	0 304.1-2 02.0	5.0	20.0			
		Quantinei				-	-	_	
		Name	IS	I ransition	Scan	Type	Precursor Ion	Product Ion	Uncertainty
		Cocaine-d3		1 307.1 -> 185.0	IVIEUVI	1510	307.1	185.0	Relative
		Qualifier							
		Precursor Ion	Product Ion	Transition	Rel. Resp.	Uncertainty	Area Sum		
		307.1	85	0 307.1 -> 85.0	3.7	20.0			

Steps	Detailed Instructions	Comments	
	 b Select Method Tasks > Calib Curve Setup, c For each target compound ch CF Origin to Force. 	ange the	

Realized Agilent MassHunter Quantitat	ive Ana	lysis - [New Method]								
Elle Edit View Analyze Method Update Report Iools Help										
🗅 😂 🖕 🖓 🖕 🖓 Analyze Batch 🥹 🛓 Layout: 🔜 🔡 🧱 🔝 🕰 🗛 Restore Default Layout										
Method Tasks ×	Meth	nod Table			_				×	
New / Open Method	. Tim	ie Segment: 👄 <all></all>	> •	⇒ Compound:	🔙 Meth	▼ 🛋 🛛 <u>R</u> eset T	able View			
Method Setup Tasks	: Lev	el Name Prefix: L	# o	f Levels: 5	<u>C</u> reate Lev	vels				
K MRM Compound Setup	Sa	mple								
K Retention Time Setup		Name	Туре	Level	Acq. Date-Time	Data File				
😥 ISTD Setup		CMAMCal_L5.d		1	ĺ	CMAMCal_L5.d]			
🚀 Concentration Setup		Quantifier								
🕂 Qualifier Setup		Name	TS	Transition	Scan	Туре	CF	CF Origin	CF Weight	
🚀 Calibration Curve Setup		Amp	1	136.2 -> 91.4	MRM	Target	Linear	Force	None	
Globals Setup		Amp-d5	1	141.1 -> 93.4	MRM	ISTD				
	-	Cocaine	1	304.1 -> 182.0	MRM	Target	Linear	Force	None	
Save / Exit		Cocaine-d3	1	307.1 -> 185.0	MRM	ISTD		-		
🕅 Validate		MDMA	1	194.2 -> 163.2	MRM	Target	Linear	Force	None	
Ng Validate		MDMA-d5	1	199.2 -> 164.3	MRM	ISTD		-		
Save Save		Meth	1	150.1 -> 119.3	MRM	l arget	Linear	Force	None	
Save As		Vietn-d5	1	155.1-> 92.3	MRM	1510				
🔀 Exit										

Steps	Detailed Instructions		Comments						
4 Validate and save the method.	a Select Save/Exit > V validate the method s	/alidate to setup.	 You can view that do occur screen. 	any validation errors at the bottom of the					
	Method Tasks ×	Method Table							
	New / Open Method	Time Segment: 🖛 <all></all>	Compound:	Meth-d5 🛛 💌 🔜 Reset					
	Method Setup Tasks	Level Name Prefix: L	# of Levels: 5	Create Levels					
	MPM Compound Setup	Sample							
	Potention Time Setup	Name	Type	Acg. Date-Time Data File					
	- ISTD Setup	CMAMCal 15 d		CMAMCal 15					
	Concentration Setup	Ouestifies		on indu_co.					
	Concentration Setup	Quantifier		(
	A Qualifier Setup	Name	TS Transition	Scan Type					
	. Calibration Curve Setup	Amp	1 136.2 -> 91.4	MRM Target					
	Globals Setup	Amp-d5	1 141.1 -> 93.4	MRM ISTD					
	Save / Evit	Cocaine Cocained3	1 304.1-> 182.0	MRM Iarget					
	Suve / Exit	MDMA	1 194 2 -> 163 2	MRM Target					
	Validate	MDMA-d5	1 199.2 -> 164.3	MRM ISTD					
	Ba Savo	Meth	1 150.1 -> 119.3	MRM Target					
	SavoAc	Meth-d5	1 155.1 -> 92.3	MRM ISTD					
	Save As	Method Error List	ent MassHunter Ouantitative A	Analysis					
	Manual Setun Tacks	Category Message	N						
		Q	Method validated. No e	rrors or warnings found.					
	Outlier Setup Tasks								
	Advanced Tasks		ОК						
	1								
	 b After the validation m click OK. c Select Save/Exit > E 	nessage appears, Exit , and click Yes							

- to the Would you like to apply this
- method to the batch? prompt.

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.

Steps					Det	ailed Instruc	tions						Comme	nts						
 Analyze the ba results for each Examine the Message(s), samples with signals. Examine the messages. 	a b c	 a Click the Analyze Batch icon a Analyze Batch i in the toolbar to start batch analysis. b Pass the mouse cursor over the quantitation message for Sample 1. c Pass the mouse cursor over the flags for the first 2 calibration standards. 								 Note that the program found no data for Amphetamine (Amp) in Sample-1. Note that two calibration standards contain outlier data. 						ds				
Outlior Flog	He Agi	ilent /	MassHunter	Quantitativ	e Analys	is - DrugsOfAbuse - iii_	Test_01													
Outlier Flag	Eile	Edit	View Ana	alyze <u>M</u> etho	d <u>U</u> pda	te <u>R</u> eport <u>T</u> ools <u>H</u> elp			e											
Messages				Analyze Batch		Layout: 📷 📷 🖾 🛄	A Resto	re <u>D</u>	efault La	ayout										
		nia							_					1						×
	Sam	nple:	<u>∎</u> <u>I</u> Sa	mple Type:	<ali></ali>	<all> Compound: 🐖 1: Amp</all>					ISTD: Amp-d5 Time Segment: <a td="" ▼<=""><td colspan="3"></td>									
Samj				le		Amp Met	_		_	_	Amp Results			Qualifi	er (119	Amp-c	15 (C	Jualifier	(124	
	۲	8	Name	Туре	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	S/N MI	RT F	Resp. F	Ratio S	/N MI
	0		Blank-1	Blank		5/12/2006 1:48 PM	1													
		4	Calib-L1 Calib-L2	Cal	L1 L2	5/12/2006 1:51 PM 5/12/2006 1:54 PM	2.5000	2.1	658 1059	49.	H	3.3187	3.3187	132.7	24.3 33.5	45.	2.1	1397	25.9 I 25.9 4	Infi L
			Calib-L3	Cal	L3	5/12/2006 1:57 PM	12.5000	2.1	2673	107		13.6808	13.6808	109.4	26.7	146	2.1	1377	26.3 4	46.
• •• •	-	+	Calib-L4 Calib-L5	Cal	15	5/12/2006 2:00 PM 5/12/2006 2:03 PM	25.0000	2.0	4952	20.	H	26.7561	26.7561	99.6	29.1	49.	1.9	1304	28.8 2	21.
Quantitation			QC-L2	QC	L2	5/12/2006 2:06 PM	5.0000	2.1	1006	81.		5.2293	5.2293	104.6	27.7	34.	2.1	1356	31.1 4	42.
Message			QC-L4 Sample-1	QC	L4	5/12/2006 2:09 PM 5/12/2006 2:12 PM	25.0000	2.1	4716	91.		27.8039	27.8039	111.2	25.6	60.	2.1	1196	31.1 9	91.
-	1		Sample-2	Sample		5/12/2006 2:15 PM		2.1	1004	80.		4.8977	4.8977		30.9	70.	2.1	1445	25.7 2	29.
			Sample-3	Sample		5/12/2006 2:18 PM		2.1	2590	74.		14.2183	14.2183		25.3	65.	2.0	1284	29.8 1	29
	Com	poun	id Informati	on	_					×	Calib	ration Curve					_	_		×
				医血金山		⇔ ‡ <u>№</u>					<u>ب</u>	Type: Lin	ear	Origin:	Force	• W	eight: N	lone 👻	ISTD	QC
	+ MRN € x10	2	0.2-> 91.4) C	www.sam_u	2.u	2.143					2 +	* ‡ 🖗 -				1.0.00				
	JNO 2	5-				Δ				AI S	mp - 5	1 y = 7.0935	els Used, 5 P * x	oints, 5 Poi	nts Use	id, 2 QCs				1
	2.2	25-								suod	1.	R^2 = 0.99	942480						/	•
	13	2-								Res	1.	4-					/	/		
	1	.5-								ative	1.	2-				/				
	1.2	25-								Rel	0	8-			/					
	0.7	75-									0.	6-		/						
	0	.5-				·····				-	0.	4-								
0.25									0.	2-	•									
	-0.2	25-										Υ <u></u>								
			1.2 1.3 1.	4 1.5 1.6 1	./ 1.8	1.9 2 2.1 2.2 2.3 2	.4 2.5 2.6 2 Acquisit	ion T	.8 2.9 ime (mir	1)		-0.2 0	0.2 0.4 0.6	5 0.8 1	1.2	1.4 1.6	1.8	2 2. Relative	2 2.4 Concer	2.6 ntration
													X 1.69 Y 12	8.17 Samp	ole-2	Amp	11	1 Sample	es (11 to	otal) .::

2 Save the batch.

- a Select File > Save Batch.
- **b** Select **File > Close** to close the batch.



Agilent 6410 Triple Quad LC/MS Familiarization Guide

Exercise 3 Review quantitation results

Task 1. Navigate the Batch Table results58Task 2. Change result window layouts63Task 3. Export and print results70

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.

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.



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, observing changes in the Batch Table and Compound Information data. It also shows you how to display various sample types.

Steps							tailed Ins	tructio	ns			Comments									
1	1 Upen the batch file <i>iii_</i> Test_01.batch.xml., created in Exercise 2.					 a to start the Quantitative Analysis b rogram, click the Quantitative c Analysis icon on your Desktop b Click Open Batch icon on the toolbar to display the Open Batch dialog box. c Navigate to \<i>Your Directory</i>\ DrugsOfAbuse and select iii_Test_01.batch.xml. 									The main View that appears should look like the one below. This is the default layout and contains the default column settings.						
			gilent	MassHunt	er Quantita	tive Ar	alysis - Drugs()fAbuse - ii	i_Test	_01										BX	
		i Eik	e <u>E</u> dit	<u>View A</u> nal	yze <u>M</u> ethod	Update	e <u>R</u> eport <u>T</u> ools	Help													
					Analyze Bate	h 🕜	Layout:			Restore <u>D</u> efaul	ít Layout										
		Ba	tch I at	xke					_			1070. Aug	-	I Thurs						×	
		: 5	ample:	T 💽 🔊	Carrela	(AII>	 Compour 	id: 💓 1: An	φ		• •	ISTD: Amp-c	5	1 Time S	egment:	<a td="" •<=""><td></td><td></td><td></td><td></td>					
		G) [2	Name	Tune	Level	Acq Date-Time	Exp. Conc.	BT	Besn	S/N M	Calc. Conc	Final Conc.	Accuracy	Batio	S/N	ы мі	Amp-o	Besn	Batio	
		٠.	0	Blank-1	Blank		05/12/2006														
		-	؟	Calib-L1 Calib-L2	Cal Cal	L1 L2	05/12/2006	2.5000	2.141 2.140	657.5479 1051.6129	49.01	3.3303	3.3303 5.7475	133.2 114.9	24.3	45.33 Infinity		2.129	1391.7148 1289.6946	26.0	
				Calib-L3	Cal	L3	05/12/2006	12.5000	2.134	2673.4935	107.59	13.6808	13.6808	109.4	26.7	145.88		2.121	1377.4550	26.3	
		-		Calib-L4 Calib-L5	Cal	L4 L5	05/12/2006	25.0000	2.022	4951.6051 18605.3105	20.20	26.7560	26.7560	107.0	29.0 27.0	49.24 39.11	\exists	1.990	1304.4692	28.8	
				QC-L2	QC	L2	05/12/2006	5.0000	2.142	1005.9952	80.96	5.2293	5.2293	104.6	27.7	34.34		2.131	1356.0175	31.1	
		-	0	QU-L4 Sample-1	Sample	L4	05/12/2006	25.0000	2.135	4715.2905	91.09	27.8011	27.8011	111.2	25.6	60.63	H	2.121	1195.5167	31.1	
			_	Sample 2	Sample		05/12/2006		2.143	1003.8094	80.42	4.8977	4.8977		30.9	70.27		2.130	1444.6758	25.7	
				Sample-3	Sample	-	05/12/2006		2.105	2589.6606	/4.8/	14.2482	14.2482		25.3	65.18		2.089	1281.1231	29.9 1	
		<							_	ш					_					>	
		Co	mpoun	d Information	n					×	Calibra	tion Curve								×	
		:		🗛 🖃 🛋	五山 金		₽ ↔ \$ ▲				:	Type: Line	ər	 Origin: 	Force	-	Weig	ht: No	ne 🔻 I	(STD QC	
		+ M	RM (136	i.2 → 91.4) CM	IAMBIk_01.d						i 🔽 \leftrightarrow	‡ 💢 •									
	1 08 07 06 05 04 03 04 03 04 03 04 03 04 03 04 03 04 03 04 12 13 14 15 15					Amp as x1 body age averav average average average average average average average ave						App - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 2 QCs						22 24	26		
	1.2 1.3 1.4 1.3 1.0						2 2.1 2.2		Acquis	ition Time (min)			5 5.0					F	telative Conv	centration	
								N				Blank-	1	Amp		11 Sam	oles (1	1 total)			
-																					

Task 1. Navigate the Batch Table results

Steps	Detailed Instructions	Comments
 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 than those you see in the figure above, 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. Restore Default Layout To restore the default column settings, right-click anywhere in the Batch Table window and select Restore Default Columns. To hide extra panes, click the highlighted icons other than the Show/Hide Chromatogram icon A 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 63.
 3 Scroll from sample to sample until you reach the end of the Batch Table, and then return to Cal-L5. Use the Next Sample and Previous Sample arrows on the toolbar . Note the changes in the Batch Table and Compound Information of amphetamine for each sample. Select sample Calib L4 in the Batch Table to view the Batch Table and Compound Information changes. 	 a Click the Next Sample arrow in the Batch Table Standard toolbar until the system displays the desired sample. Inspect the changes in the Compound Information window. b To return to Cal-L5, click the Previous Sample icon in the Batch Table Standard toolbar. c 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.

Task 1. Navigate the Batch Table results

Steps	Detailed Instructions	Comments
 4 Scroll from compound to compound through all four compounds. Use the Next Compound and Previous Compound arrows on the toolbar. Compound: 1: Meth 	 a Click the Next Compound or Previous compound arrow in the toolbar until the system displays the desired compound. b Inspect the changes in Batch Table, Compound Information and Calibration Curve windows. 	
 Review the differences in the Batch Table, Compound Information and Calibration Curve windows between the compounds. Select Cocaine from the list. 	c Click the down arrow next to the Compound list.d Select Cocaine.	

Task 1. Navigate the Batch Table results

St	ieps	D	etailed Instructions	Comments		
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. 	a b	Click the Multiple Compound View icon in the toolbar to display the quantitation results for all target compounds. You can also the View > Batch Table Layout > Multiple Compound View menu item. Click the Cal-L4 cell, and note the difference in RT in the Compound Information window for each	A different set of columns is displayed when you are in Multiple Compound View mode versus Single Compound View mode. If you add a column to the table when you are Multiple Compound View mode, that change is not automatically made in the Single Compound View mode.		

📆 Ag	Aglient Massifunter Quantitative Analysis - DrugsOfAbuse - iii_Test_01																
<u>E</u> ile	Elle Edit View Analyze Method Update Report Tools Help																
101	🗅 🧽 🚽 🕼 💭 Analyze Batch 🛛 🐠 📜 Layout: 🧱 🔡 🛗 🔝 🕼 🖾 Restore Default Layout																
Bate	Batch Table x																
Sar	Sample: 👔 🖟 Sample Type: <all> 👻 Compound: 🖬 1: Cocaine 🔹 🐨 ISTD: Cocaine-d3 Time Segment: <all th="" 🂝="" 🌪="" 👯="" 💝="" 💽="" 🛄="" 🦉<=""></all></all>																
			Sample	e			Amp Results	3		Meth Resul	ts		MDMA Resu	Ilts		Cocaine Res	ults
٢	Vame Type Level Acq. Date-Time				Acq. Date-Time	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy	RT	Final Conc.	Accuracy
0	٣	Blank-1	Blank		5/12/2006 1:48 PM				1.338	8.0617		2.466	6.9724		2.433	11.8235	
	٣	Calib-L1	Cal	L1	5/12/2006 1:51 PM	2.141	3.3187	132.7	2.247	2.5936	103.7	2.276	2.2824	91.3	2.453	2.3087	92.3
	7	Calib-L2	Cal	L2	5/12/2006 1:54 PM	2.140	5.7493	115.0	2.248	5.1011	102.0	2.277	4.6561	93.1	2.454	4.2682	85.4
	٣	Calib-L3	Cal	L3	5/12/2006 1:57 PM	2.134	13.6808	109.4	2.247	15.1623	121.3	2.277	11.2728	90.2	2.459	11.5607	92.5
•		Calib-L4	Cal	L4	5/12/2006 2:00 PM	2.022	26.7561	107.0	2.228	27.2574	109.0	2.264	24.8702	99.5	2.449	25.2511	101.0
		Calib-L5	Cal	L5	5/12/2006 2:03 PM	2.101	124.4844	99.6	2.237	124.2764	99.4	2.271	125.1668	100.1	2.448	125.0768	100.1
		QC-L2	QC	L2	5/12/2006 2:06 PM	2.142	5.2293	104.6	2.248	5.2414	104.8	2.276	4.8567	97.1	2.453	4.2831	85.7
		QC-L4	QC	L4	5/12/2006 2:09 PM	2.135	27.8039	111.2	2.246	27.7713	111.1	2.276	23.0331	92.1	2.455	24.5377	98.2
0	7	Sample-1	Sample		5/12/2006 2:12 PM				2.568	4.4257		2.315	5.6138				
		Sample-2	Sample		5/12/2006 2:15 PM	2.143	4.8977		2.250	5.8102		2.280	5.1778		2.460	4.3735	
		Sample-3	Sample		5/12/2006 2:18 PM	2.105	14.2183		2.236	14.1876		2.267	10.7772		2.446	10.9299	

c To return to the display of detailed quantitation results for the selected target compound, click the **Single** Compound Display icon in the

I	

compound, click the Single
 Compound Display icon in the toolbar.
 d If necessary, click the down arrow next to the Compound list, and select

Cocaine.

compound.

Task 1. Navigate the Batch Table results

Steps	Detailed Instructions	Comments
 6 View selected Sample Types. Display only the Calibration standards. Then display all Sample Types. 	 a Click the down arrow in the Sample Type dropdown list. The Sample Type dialog box is displayed. b Clear the <aii> check box and mark the Cal check box.</aii> 	
	 Elle Edit View Analyze Method Update Report Tools ¹ 	
	Batch Table	
	Sample: 👔 🚛 Sample Type: <all> 🔻 Compound:</all>	
	Sample Type ≤ ③ ▼ All> ● ▼ Calib-L ● Calib-L ○ ● Allb ● Calib-L ● MatBlk □ TunChk Sample ○ ● Calib-L ● Calib-L ● Calib-L ● NatBlk □ Calib-L ● Calib-L ● Calib-L ● Calib-L	
	c Click OK.	
	The Batch Table should contain only	
	d Click the down arrow in the Sample	
	Type dropdown list.	
	e Click <aii>, then click OK.</aii>	
	The system marks all the check boxes	
	and displays all sample types.	

Task 2. Change result window layouts

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

S	teps	Detailed Instructions	Comments
1	Use layout icons on the toolbar to position the Batch Table, Compound Information and Calibration Curve windows. • The default layout is called Table Top because the Batch table is at the top of the main view. • Change the layout to Table Left, then to Table Right. • Return to the Table Top layout.	 a Click the Lavout – Table Left icon in the toolbar b Click the Layout – Table Right icon in the toolbar c Click the Layout – Table Top icon 	
2	Use layout icons on the toolbar to maximize each individual window: Table Compound Information Calibration Curve Return to the default layout.	 a Click the Maximize Table icon in the toolbar b Click the Maximize Compound Information icon in the toolbar c Click the Maximize Calibration Curve icon in the toolbar d To return to the default layout, click the Restore Default Layout icon on the toolbar. 	
3	Change the panes in the Compound Information window for Cal-L4. • Show qualifiers. • Show spectra. • Show ISTD chromatogram, qualifiers and spectra.	 a In the Batch Table, select the Cal-L4 row. a In the Compound Information toolbar, click the Show/Hide Qualifiers icon	 This step assumes that you started this task with just the Chromatogram pane in the Compound Information window. 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.

Task 2. Change result window layouts



- 4 Save the default layout without the calibration curve.
 - Save the new layout as Batch Table plus Compound Information in the DrugsOfAbuse folder.
- a Close the Calibration Curve window.
- b Select View >Window Layout > Save Layout.

The system displays the Save Layout File dialog box.

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

St	eps	De	etailed Instructions	Com	ments
5	 Load the newly created layout. Restore the default layout. Load the layout Batch Table plus Compound Information. 	a b	Click Restore Default Layout on the toolbar. Select View > Window Layout > Load Layout . The system displays the Load Layout dialog box.		
		C	Lookin: Image: DrugsO/Abuse Image: DrugsO/Abuse Image: DrugsO/Abuse		
			Information and click Upen . The results window should now look like Figure 8 on the next page		



Figure 8 Results window

St	eps	3					Detail	ed Ins	structio	ons	Comments
6	Cr Fig ca in Hi or	eate the gure 9 or libration formatio nt: More the left	layou n page n curve n wind e than	t as 67, and dows the l	shown in with the I compound s floating. Batch Table	Detailed Instructions Comments n in a Restore the default layout (click Restore Default Layout on the toolbar). Bight-click inside the title bar of the Calibration Curve window, and then mark the Floating check box. Image: State St					
							Calibrati Calibrat	n Curve Type: Line	ck the t nd Info k the F ne wind Figure	e itle rma loat low: 9.	bar of the tion window, and ting check box. s to match the
	Agile	nt MassHunter	Quantitat	ive Ana	lysis - DrugsOfAbuse	e - iii_Tes	L_01		- 0	×	Calibration Curve x
: E	ile 🛿	dit ⊻iew <u>A</u> n: U⊒llonalic⊒i	alyze <u>M</u> eti Analyze Bat	hod Up ch	Eavout:	i <u>H</u> elp ₩ IIII (∧)	Restor	e Default L	avout		- Type: Linear • Origin: Force • Weight: None • ISTD QC
в	atch	Table						-		×	₩ + ‡ ¾ -
1	Samp	le: 👔 🚺 Sa	mple Type	<a -<="" td=""><td>Compound: 🔙 1:</td><td>•</td><td>STD:</td><td></td><td>R 🖌 🖌 🖉</td><td></td><td>Cocaine - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 2 QCs \$ x10 1 J y = 5.5508 * x</td>	Compound: 🔙 1:	•	STD:		R 🖌 🖌 🖉		Cocaine - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 2 QCs \$ x10 1 J y = 5.5508 * x
			Samp	le		Cocain	ə		Cod	aine	⁸ 1.2- R ² = 0.99985846
0	D	♥ Name	Туре	Level	Acq. Date-Time	Exp. Co	nc. RT	Resp.	S/N MI	Cal	
	0	P Blank-1	Blank		5/12/2006 1:48 PM		2.433	20	1.25		-3.0 ਵ
		Calib-L1 Calib-L2	Cal	L1 L2	5/12/2006 1:51 PM 5/12/2006 1:54 PM	2.5	2.453	9716	81.20		0.4-
•		Calib-L3 Calib-L4	Cal Cal	L3	5/12/2006 1:57 PM 5/12/2006 2:00 PM	12.5 25.0	000 2.459	25187 50649	103.81		0-
		Calib-L5	Cal	L5	5/12/2006 2:03 PM	125.0		199967	98.38	\square	-0.2 0 0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6
	-	QC-L4	QC	L4	5/12/2006 2:09 PM	25.0	000 2.455	48582	93.16	F	Relative Concentration
	U	Sample-1 Sample-2	Sample Sample		5/12/2006 2:12 PM 5/12/2006 2:15 PM		2.460	9735	97.71		Compound Information
		Sample-3	Sample		5/12/2006 2:18 PM		2.446	24841	93.30		: 1
_ <										>	+ MRM (304.1 \rightarrow 182.0) CMA. $g \times 10^{4}$ 2.449 $g \times 10^{4}$ 2.449 $g \times 10^{4}$ 2.449 $g \times 10^{2}$ Ratio 3.9 $g \times 10^{3}$ 182.0 $g \times 10^{2}$ Ratio 3.9 $g \times 10^{3}$ 182.0 $g \times 10^{3}$ 185.0 $g \times 10$
			 Detailed Instruction te the layout as shown in e 9 on page 67, with the ration curve and compound mation windows floating. More than the Batch Table is e left. Right-click inside Calibration Curve mark the Floating Right-click the thi Compound Information then mark the Floating Right-click the thi Compound Information then mark the Floating Resize the windor layout in Figure 9 		Acquisition Time (mi Acquisition Time (mi Mass-to-Charge (m/z)						

Figure 9 Display with Calibration Curve and Compound Information windows floating

Steps	Detailed Instructions	Comments
	e Right-click inside the title b	par of the
	Compound Information wir	ndow, and
	then clear the Floating che	eck box.
	f Resize the windows to mat	tch the
	layout in Figure 10.	





Steps	Detailed Instructions	cructions Comments k inside the title bar of the n Curve window, and clear ng checkbox. ng checkbox. Compound Information o that the layout corresponds e pictured at the start of the • You must anchor the Calibration Curve window first, and then the Compound Information window, to recreate the default layout. the program main View. • You must 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, you can right-click the title bar of the Calibration Curve window and drag it to the right. A gray rectangle is drawn that shows where this window will be placed within the main view. Drag the Calibration Curve to the bottom right corner of the main view.	Comments				
	 g Right-click inside the title bar of the Calibration Curve window, and clear the Floating checkbox. h Move the Compound Information window so that the layout corresponds to the one pictured at the start of the task. 						
 7 Recreate (do not restore) the default layout. In this step you learn to recreate layouts without using the layout icons or Restore Default Layout. 	a Maximize the program main View.	 You must 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, you can right-click the title bar of the Calibration Curve window and drag it to the right. A gray rectangle is drawn that 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

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.

Steps	Detailed Instructions	Comments
 Export the batch file <i>iii</i>_Test_01. Specify My Documents as the destination directory. Use <i>iii</i>_Test_01.xls as the export file name, where "<i>iii</i>" are your initials. 	 a To make the Batch Table window active, click the title bar of the Batch Table window. b Select File > Export > Export Table. c Select My Documents as the destination directory. d Enter <i>iii</i>_Test_01.xls as the export file name. e Click Save. 	

F	🖥 Ag	ilent MassHunter Quantitative Analysis - DrugsOfAbuse -	iii_Test_01												
1	<u>F</u> ile	Edit View Analyze Method Update Report Tools	<u>H</u> elp												
1	Ð	New Batch	Ctrl+N	estore	Default L	ayout									
	Þ	Open Batch	Ctrl+0	Ctrl+O											
		<u>S</u> ave Batch	Ctrl+S			-	IST	D: Cocaine	Time Segmer	nt: <a th="" 👻<=""><th></th><th>*</th><th>1</th><th></th>		*	1		
Γ		Save Batch As					Coc	aine Results			Qualifi	er (82.0)	R.	Coc	
F		Close Batch						(_	
		A <u>d</u> d Samples		RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	S/N	MI	RT	
		Export	+	E	xport Tab	le		11.8235	11.8235					2.40	
L	6							2.3087	2.3087	92.3	3.7	Infinity		2.45	
		Page Setup		E	xport <u>G</u> ra	pnics		4.2682	4.2682	85.4	3.9	Infinity		2.45	
	8	Print	Ctrl+P	2.459	25187	103.81		11.5607	11.5607	92.5	3.9	104.51		2.45	
L	R.	Print Preview		2.449	50649	118.29		25.2511	25.2511	101.0	3.9	354.91		2.44	
	-			2.448	199967	98.38		125.0768	125.0768	100.1	3.8	90.77		2.44	
		1: C:\QuantData\DrugsOfAbuse\iii_Test_01.batch.xml		2.453	9246	83.17		4.2831	4.2831	85.7	3.5	42.17		2.45	

Figure 11 Export results

- **2** View the batch results as they
- a Start Microsoft Excel.
- appear in Excel; then exit Excel.
- **b** Open **My Documents***iii*_Test_01.xls.
- Note what is exported and what is not.
- **c** Note what is exported and what is not.
- **d** Close Excel when you are finished.

Task 3. Export and print results

tep	S						Detailed In										
📧 Mi	icros	oft l	Excel - iii_	Test_01.	xls										[×
	Eile	E	dit <u>V</u> iew	Insert	Format	<u>T</u> ools <u>D</u> ata <u>W</u> ind	ow <u>R</u> eport Design	Help Adob	e PDF				Туре	a question for	help 👻	_ 8	×
					149	🚆 Arial	▼ 10 ▼	BIU	%, *:% \$?? 掌 ≇ ⊞ • 🆄 • A								
-73	Pro	ess	Report 🛐	Clear Re	suits 🕈	Add Data 🖣 Add G	Fraphics 👫 Add Fo	rmatting 📯 /	Advance	d Properti	es 🚺 🔪	alidati	e Design 🌀	@ _			
100.200	_	F16	6	•	fx								1111				
1	A	В	C	D	E	F		G	Н	1	J	K	L	М	N	0	
1						Sample		Cocaine Method				Cod	aine Results			Qualifi	
2			Name	Туре	Level	Acq. Da	ite-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calo, Cono.	Final Conc.	Accuracy	Ratio	Ĺ
3	Amp	Coca	Blank-1	Blank		2006-5-12 1:48 PM			2.43335	19.782	1.2485043	FALS	11.82345009	11.82345009			Ĺ
4			Calib-L1	Cal	L1	2006-5-12 1:51 PM		2.5	2.45262	5188.63738	72.45171	FALS	2.308652511	2.308652511	92.34610043	3.7181	
5			Calib-L2	Cal	L2	2006-5-12 1:54 PM		5	2.454	9716.41592	81.202101	FALS	4.26823458	4.26823458	85.36469159	3.89733	
6			Calib-L3	Cal	L3	2006-5-12 1:57 PM		12.5	2.45898	25186.8579	103.80841	FALS	11.56066094	11.56066094	92.4852875	3.91193	
7			Calib-L4	Cal	L4	2006-5-12 2:00 PM		25	2.44872	50648.7022	118.28977	FALS	25.251111	25.251111	101.004444	3.85454	
8			Calib-L5	Cal	L5	2006-5-12 2:03 PM		125	2.44818	199966.716	98.383157	FALS	125.0768093	125.0768093	100.0614474	3.80143	I
9			QC-L2	QC	L2	2006-5-12 2:06 PM		5	2.45348	9246.17986	83.172903	FALS	4.28310766	4.28310766	85.6621532	3.45981	
10	-		QC-L4	QC	L4	2006-5-12 2:09 PM		25	2.45503	48581.9897	93.164298	FALS	24.53773699	24.53773699	98.15094798	4.01757	
11	Amp	d5: Int	Sample-1	Sample		2006-5-12 2:12 PM				-	-	FALS					i l
12	-		Sample-2	Sample		2006-5-12 2:15 PM		-	2.45998	9735.0305	97.707275	FALS	4.373450699	4.373450699		3.59577	I
13			Sample-3	Sample		2006-5-12 2:18 PM			2.44552	24840.7796	93.299855	FALS	10.92988924	10.92988924	×	3.924	
14	-														-		1

Figure 12 Batch table in Excel

- **3** Preview printouts for Batch Table and Compound Information data.
 - Print the Batch Table and Compound Information.
 - Save and exit the batch if you are not going to perform Exercise 4 right away.
- a Click inside the title bar of the Batch Table window, and select File > Print Preview.
- **b** Inspect the display of the Batch Table in the **Print Preview** window to make sure it looks the way you want it.
- c Close the Print Preview window.
- d When the Batch Table is satisfactory, select File > Print.
- e Repeat steps a-d for the Compound Information.
- f If you are not moving on to Exercise 4, select File > Save Batch.
- g Select File > Exit.

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

Task 3. Export and print results


Agilent 6410 Triple Quad LC/MS Familiarization Guide

Exercise 4 Use three new tools to evaluate results

Task 1. Adjust the calibration curve fit74Task 2. Integrate without parameters77Task 3. Detect outliers89

In this exercise you use three new 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
- Parameter-less 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

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.



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 re-analyze the batch.

Si	ieps	De	etailed In	structio	ons		(Comments								
1	If necessary, open the batch file <i>iii_Test_01.batch.xml</i> . If the batch is already open, skip to step 2.	 a To start the Quantitative Analysis program, click the Quantitative Analysis icon on your Desktop. b Click Open Batch alog on the toolbar to display the Open Batch dialog box. c Navigate to \Your Directory \DrugsOfAbuse and select iii_Test_01.batch.xml. You can also selecting P MassHunt Quantitating Start menu. If the defau click Restor toolbar before 										To access the program by Programs > Agilent > er Workstation > ve Analysis from the I. ult layout is not present, pre Default Layout on the fore opening the batch. re Default Layout				
2	 Find the accuracy outlier for amphetamine, and change the curve fit. Set Origin to Ignore, and Weight to 1/y. 	a	Make su single c the disp See circ below.	ure the l ompour layed ta led port	Batcl Id dis rget ions	n Table is set t splay mode, ar compound is A of the illustrat										
		Co	mpound: 🔙	1: Amp		💌 🔿 IST	D: Amp-d5	p-d5 Time Segment: <all></all>								
		b	 Point to the cell in the Calib-L1 row and the Accuracy column to display the Outlier message as shown below. Cells con- red (high 									containing outliers can be in gh) or blue (low).				
		itch	Table													
		amp	ile: 👔 🚺 🕴 Sa	ample Type:	<ali></ali>	🔹 🗌 Compound: 💷 1	: Amp		-	ISTE	D: An	np-d5 Tim	e Segment:	<a td="" •="" 🔲<="">		
		_		Samp	le		Amp Met				A	mp Results				
)	P Name	Туре	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy		
		•	Plank-1	Cal	L1	5/12/2006 1:48 PM	2.5000	2.141	658	49.10		3.3187	3.3187	132.7		
			V Outlier(s)				2.140	1059	42.25		5.7493	5.7493	115.0		
			Amp: Accura	icy value = 13	2.7 is ou	Itside the allowed range [80.0, 120.0]	2.134	2673	20.26	H	13.6808	13.6808	109.4		

Task 1. Adjust the calibration curve fit

Steps	Detailed Instructions	Comments
	c 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 ♥ ♥ Type: Linear ♥ 0rigin Include ♥ 1000	 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). 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.
3 Analyze the batch and inspect the results in the Batch Table.	 a Click the Analyze Batch icon in the toolbar The toolbar to analyze the batch. b Inspect the results in the Batch Table after batch analysis. . Accuracy 97.2 97.3 102.6 103.7 99.2 36.9 107.9 	
4 Find accuracy outliers, if any, for other compounds.	 a Click Next Compound in the Batch Table toolbar to view individual compounds, such as Cocaine, MDMA, and Met. b 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.

Task 1. Adjust the calibration curve fit

Steps			etailed Instructions	Comments			
5	Change the curve fit for methamphetamine, and analyze the batch.	a b	In the Calibration Curve Fit window, set Origin to Ignore , and Weight to 1/y . MassHunter displays a revised curve fit formula and R2 value. Click Analyze Batch in the toolbar Gist Analyze Batch to analyze the batch. The Batch Table displays the new results after batch analysis				

Task 2. Integrate without parameters

This section 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

Criteria Dil. High Conc.

Dil. Pattern Extract Left m/z Extract Right m/z

Fragmentor High m/z HitlD

- Closely examine the chromatogram, looking for such details as:
 - outlier messages
 - baseline parameters
 - peak labels

St	teps	Detailed Instructions	Comments
1	 Add integration columns to the Batch Table. Add the Integrator Type and Integrator Parameters columns from the Compound Method list. Add the Integrator Metric column to the Batch Table from the Compound Results list. 	 a Right-click anywhere in the E Table, and select Add/Remove Columns. The system displays the Columbia dialog box. b Select Compound Method from Select Columns From dropdo c Select Int. (Integrator Type) a Parms. (Integrator Parameter the Available Columns list, a Add. The Quantitative Analysis promoves the selected columns in the original sector of the selected columns of the selected columns of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the selected columns in the original sector of the sector of	 This task assumes that the batch, <i>iii_Test_01</i>, is already open. If it is not, see step 1 in Task 1. mns m the wn list. nd Int. s) from nd click gram to the wrder list.
		Columns	
		Select Columns Erom:	
		Compound Method	
		Available Columns:	Show these columns in the order:
		Cmpd. Group Ad	Exp. Conc.

Add All ->>

<-- Remove All

Task 2. Integrate without parameters

Steps	Detailed Instructions	Comments
	d Select Compound Results Select Columns From dro e Select Int. Metric (Integra from the Available Colum click Add. The system moves the se column to the Show thes the order list. f Click OK.	s from the pdown list. ator Metric) i ns list, and lected e columns in
	Columns	
	Select Columns Erom:	
	Compound Results	
	Available Columns:	Show these columns in the order:
	Height Int End Int. Metric Flag Int. Start ISTD Conc. Ratio ISTD Conc. Ratio ISTD Resp. Ratio Matrix Spike % Dev. Matrix Spike % Recovery Q. Computed	Add -> RT Resp. S/N Mi Cale: Conc. Final Conc. Final Conc. Accuracy Int. Metric

- **2** View the default integration values for amphetamine.
 - View the Int. type and Int. Parms. columns
 - View the Int. Metric column.
- a Click **Previous Compound** in the Batch Table toolbar <u>a</u> to view amphetamine (**Amp**),
- **b** Examine the default values in the Int. and Int. Parms columns in the Batch Table.

• Note that the default 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	

eps	Detailed I	Detailed Instructions							Comments				
	c Examir Metric	ne the default v column in the	alues Batch	• These values reflect the default integration quality metric used for the target compound Amp .									
	1: Amp	🝷 📑 ISTD	: Amp-d	5			Time Seg	ment: <all></all>	-	🥞 😽 🖌			
		Amp Method					Amp Res	ults		-			
	Sample	Int. Int. Parms	RT	Resp.	S/N	м	Calc. Conc.	Final Conc.	Accuracy	Int. Metric			
		MS-MS											
	2.5000	MS-MS	2.141	658	49.10		2.4296	2.4296	97.2	Accepted			
	5.0000	MS-MS	2.140	1059	42.25		4.8673	4.8673	97.3	Accepted			
	12.5000	MS-MS	2.134	2673	107.28		12.8217	12.8217	102.6	Accepted			
	25.0000	MS-MS	2.022	4952	20.26		25.9349	25.9349	103.7	Accepted			
	125.0000	MS-MS	2.101	18605	47.90		123.9465	123.9465	99.2	Accepted			
	5.0000	MS-MS	2.142	1006	81.00		4.3457	4.3457	86.9	Accepted			
	25.0000	MS-MS	2.135	4/16	91.48	H	26.9858	26.9858	107.9	Accepted			
		MS-MS	2 1/3	1004	80.65	H	4 0131	4.0131		Acconted			
			2. 14J	1004	00.00		4.0131	+.0131		Accepted			

Task 2. Integrate without parameters

St	eps					Deta	iled Instruct	ions				Comr	nents				
}	View integration cocaine and MDI • Enlarge the ch portion of Com Information so	probl MA. romat poun that	em togi d onl'	s for ram y the		a C b T o c	lose the Calil o enlarge the n the Compo lick the Shov	oration (chroma und Info v/Hide \$	Curve w itogram irmation Spectru	indow. portion toolba m icon	ı ır,						
	quantifier and chromatogram	nd qualifier rams appear. tlier messages at the n of the Int. Metric 1 the Blank-1 sample			the	Com	pound Information	, <mark>∑∭</mark> ≸	‡ <u>^</u>								
	intersection of column and th				c iple.	+ м с А	M (304.1 -> 182.0) Iso click the	Show/I	ow/Hide Spe Hide IST	otrum ^{0.0}	, 82.0						
					•	d C	lick the Next	Compo	und ico	n in the)						
						В	atch Table to	ol bar 🝙	🛛 until tl	he							
						S	ystem display	/s the co	ompoun	d							
						C	ocaine.			• • •							
						e S	elect the Bla	nk-1 ro	N, and p	oint to							
						u T	he system di	column solavs a	nv outli	L FOW. or							
							ne system ur	spiays a	ny outin	CI							
						m	, nessage for th	nat data	as wel	l as the	•						
						n ir	nessage for tl ntegrated chr	nat data omatogi	, as wel ram for (l as the cocain	e e.						
						m ir	nessage for the sage for the sage for the sage for the sage for the same same sage for the same same same same same same same sam	nat data omatogi	, as wel ram for (l as the cocain	e.						
		Hi Agile	nt Ma	issHunter	Quantitativ	rr ir • Analys	nessage for th ntegrated chr	nat data, omatogi _Test_01	, as well ram for (l as the cocain	e.						
		Hin Agile	nt Ma Edit L	issHunter ⊻iew <u>A</u> na	Quantitativ alyze <u>M</u> etho Analyze Batch	m ir e Analys d <u>U</u> pda	nessage for th ntegrated chr is - DrugsOfAbuse - iii te Report Iools He Layout: 记 🕅 🕅	nat data omatogi _Test_01 ₽ 	, as well ram for (l as the cocain	9 8.	_		_			
		His Agile	nt Ma ≣dit <u>\</u> Table	issHunter ⊻iew Ana La (J≣ ≰	Quantitativo alyze <u>M</u> etho analyze Batch	m ir e Analys d <u>U</u> pda	nessage for tl ntegrated chr is - DrugsOfAbuse - iii te Report Tools He Layout: 🔐 😰 🛒	nat data omatogi _Test_01 p [] 🕅 🔀 Restu	, as well ram for (ore <u>D</u> efault Lay	l as the cocain) 9.						
		File E Batch	nt Ma dit <u>v</u> Table le: P	ssHunter View Ana La (I g)	Quantitativ alyze <u>M</u> etho inalyze Batch mple Type:	rr ir e Analys d Upda	is - DrugsOfAbuse - iii te Report Tools He Layout: (2) (2) (2) (2) (2) (2) (2) (2) (2) (2)	_Test_01	, as well ram for (l as the cocain ^{/out}	e.	e-d3		Time Segment:	<4 - 10) • •	
		Agile	nt Ma Edit \ Table le: P	issHunter View Ana Ia (II 4 2 2 3	Quantitativ alyze Metho inalyze Batch mple Type: Samp	m ir e Analys d Upda d Upda <all></all>	is - DrugsOfAbuse - iii te grated chr is - DrugsOfAbuse - iii te Report Tools He Layout: The Report Tools He Compound: Report Tools He	_Test_01	, as well ram for o ore <u>D</u> efault Lay Coccine Metho	l as the cocain ^{rout}	e.	ə-d3		Time Segment: Cocaine Re	<a c<="" td="" v=""><td></td><td></td>		
		Agile Elle E Batch Sampl	nt Ma Edit V Table le: T	ssHunter ⊈iew Ana In (II 4 IN Sa Name Bank-1	Quantitativ alyze Metho analyze Batch mple Type: Samp Type Blank	rr ir e Analys d Upda d Upda (All> ble Level	Acq. Date-Time	Test_01	, as well ram for o ore <u>D</u> efault Lay Coccaine Metho Int.	vout d I as the cocain vout I I I I I I I I I I I Parms.	e. E. Cocaine RT	e-d3 Resp.	S/N M	Time Segment: Cocaine Re Calc. Conc. 11 8235	<a ¥<br="">sults Final Conc.	Accuracy	Int. Me
		Agile	nt Ma Edit \ Table le: 1 E	ISSHUNTER View Ana I I I I I I I I I I Sa Name Blank-1 Calib-L1	Quantitativi alyze Metho analyze Batch mple Type: Samp Type Blank Cal	Analys d Upda (All> Level L12	Acq. Date-Time 5/12/2006 1-31 PM	Test_01	, as well ram for (ore Default Lay Coceine Metho Int.	rout I as the cocain rout Int. Parms. Outlier(s)	 Cocain RT 2.433 	e-d3 Resp. 20	S/N M 1 25	Time Segment: Coceine Re Calc. Conc.	<a sults Final Conc. 11 8225</a 	Accuracy	Int. Mee
		Agile Ele Batch Sample	nt Ma Edit V Table le: P % E C C C C	SSHunter View Anz La CI Z Name Slank-1 Calib-L1 Calib-L2 Calib-L2 Calib-L3	Quantifativi alyze Metho analyze Batch mple Type: Samp Type Blank Cal Cal Cal	All>	Acq. Date-Time 5/12/2006 1:51 PM 5/12/2006 1:51 PM 5/12/2006 1:51 PM 5/12/2006 1:51 PM 5/12/2006 1:51 PM 5/12/2006 1:51 PM	Test_01	, as well ram for o ore Default Lav Cocaine Metho Int. MS-MS MS-MS Co MS-MS Co	rout Termina Int. Parms. Outlier(s) caine: Integr	Cocaine RT 2.433 ator four 2.459	e-d3 Resp. 20 d the follo 25167	S/N M 1251 wing proble	Time Segment: Cocaine Re Cale: Conc. 11 823 (s) with the p	<a concerning="" of="" set="" td="" the="" the<=""><td>Accuracy 2.433: Merge</td><td>Int. Me Inspect</td>	Accuracy 2.433: Merge	Int. Me Inspect
		Batch Sample	nt Ma Edit V Table	SSHunter View Ana Call () A Name Slank-1 Calib-L1 Calib-L1 Calib-L2 Calib-L3 Calib-L4 Calib-L4	Quantifativi alyze Metho analyze Batch mple Type: Samp Type Blank Cal Cal Cal Cal Cal	Analys d Upda d Upda cAll> Level L1 L2 L3 L4 L4	Acq. Date-Time 5/12/2006 1:51 PM 5/12/2006 1:57 PM	Test_01	, as well ram for o ore Default Lay Cocaine Metho Int. MS-MS MS-MS KS-MS Coc	as the cocain rout 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	 Cocain RT 2.459 2.449 2.449 	e-d3 Resp. 201 d the follo 25187 50649	S/N M 1 251 103.81 118.29 9 29 20	Time Segment: Cocaine Re Cale: Conc. 11 8245 11 59072 25 2511	<a indicate="" indicate<="" td=""><td>Accuracy</td><td>Int. Mel</td>	Accuracy	Int. Mel
		Image: Agrille Image: Elle	nt Ma Edit V Table le: P C C C C C C C C C C C C C C C C C C C	ssHunter View Ana Name Slank-1 Calib-L1 Calib-L2 Calib-L3 Calib-L4 Calib-L5 Cal-L5	Quantitativ alyze Metho malyze Batch mple Type: Samp Cal Cal Cal Cal Cal Cal Cal Cal Cal Cal	m ir	acssage for tl httegrated chr is : DrugsOfAbuse - iiii is : Beport I zols He Layout: : :::::::::::::::::::::::::::::::::	nat data, omatogi [rest_01] [p] [△] [○] Rest [△] [○] Rest [□] [○] [○] [○] [○] [○] [○] [○] [○] [○] [○	, as well ram for of ore Default Lay Coceine Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	as the cocain out 1 stD 1 stD	 Cocain RT 2.433 2.449 2.449 2.448 2.448 2.448 	e-d3 Resp. 20 d the follo 25187 50649 199967 9246	S/N M 1 25 103.81 118.29 98.38 33.17	Time Segment: Cocaine Re Cale: Conc. 11 8225 m(s) with the p 71 826 (s) with the p 72 5251 125 0768 4 2823	<a Suits Final Conc. 11 8245 Prinal Conc. 11 8245 25 2511 25 2511 25 2511 25 2513 25 2511 25 251 25 25 25 25</a 	Accuracy 2.433: Merge 101.0 100.1 85.7	Int. Me Inspect
		Image: Agile Eile Batch Batch Sample	nt Ma Edit V Table le: 1 C C C C C C C C C C C C C C C C C C C	ssHunter view Ana Name Slank-1 Calib-L1 Calib-L2 Calib-L3 Calib-L3 Calib-L4 Calib-L5 QC-L4 QC-L4	Quantitativ alyze Metho mple Type: Samp Type Blank Cal Cal Cal Cal Cal Cal Cal Cal	Image: Constraint of the second sec	acsage for the segret of the segret of the segret for the	Test_01	, as well ram for of ore Default Lay MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	vout	 Cocain RT 2.459 2.449 2.443 2.453 2.453 	e-d3 Resp. 20 d the follo 25167/ 50649 199967 9246 48582	S/N M 1 25 118 29 98.30 83.17 93.16	Time Segment: Cocaine Re Cocaine	<a< td=""><td>Accuracy 2433: Mergy 92.5 101.0 100.1 85.7 98.2</td><td>Int. Me Accept Accept Accept</td></a<>	Accuracy 2433: Mergy 92.5 101.0 100.1 85.7 98.2	Int. Me Accept Accept Accept
		Image: Agile Eile Batch Batch Sample	nt Ma Edit V Table le: P C C C C C C C C C C C C C C C C C C C	ssHunter View Ana Calip () () Name Stank-1 Calib-L1 Calib-L2 Calib-L2 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L4 Calib-L5 Cal-L4 Calib-L5 Cal-L4 Calib-L5 Cal-L4 Calib-L5 Cal-L4 Calib-L5 Cal-L4 Calib-L5 Cal-L4 Calib-L5 Cal-L4 Calib-L5 Cal-L4 Calib-L5 Cal-L4 Calib-L5 Cal-L4 Cal-L4 Calib-L5 Cal-L4 C	Quantitativ alyze Metho mple Type: Samp Type Blank Cal Cal Cal Cal Cal Cal Cal Cal Cal Cal	m in 2 Analys 4 Upda 4 Upd	acssage for the segret of the segret construction is DrugsOfAbuse - iii is Report Tools He construction Layout: Image: Compound: Image: Compound: Image: Compo	Test_01	, as well ram for of ore Default La Cocaine Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	rout	Cocain RT 2 433 2 449 2 443 2 443 2 443 2 445 2 445 2 445 2 445	e-d3 Resp. 20 d the follo 25167/ 50649 199967 9246 48582 9235	S/N M 1 25 103.61 L 118.29 98.33 [93.16 L 97.71]	Time Segment: Coccaine Re Calc. Conc. 11 8245 (1) 50768 4 2831 24 5377 24 5377 24 5377	A	Accuracy 2.433: Merge 92.5 101.0 100.1 85.7 98.2	Int. Me Instact Accept Accept Accept Accept Accept
		Agile File E Batch Sampl	nt Ma Edit V Table le: • C C C C C C C C C C C C C C C C C C C	ssHunter (iew Analysis) (if is a Name Samb-1 Samb-1 Samb-1 Samb-2 Sample-2 Sample-3 Sample-3 Sample-3	Quantitativ alyze Metho mpleyze Batch Type Blank Cal Cal Cal Cal Cal Cal Cal Cal Cal Cal	m ir	Acq. Date-Time 5/12/2006 1:3 PM 5/12/2006 2:0 PM 5/12/2006 2:0 PM 5/12/2006 2:0 PM 5/12/2006 2:0 PM 5/12/2006 2:0 PM 5/12/2006 2:0 PM 5/12/2006 2:0 PM 5/12/2006 2:1 PM 5/12/2006 2:1 PM 5/12/2006 2:1 PM	Test_01	, as well ram for of are perault La cocaine Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	vout	 Cocain RT 2 433 2 449 2 443 2 445 2 455 2 460 2 446 	e-d3 Resp. 201 dthe follo 25167 50649 199967 9246 48562 9235 24841	S/N M 125 13831 E 13839 S 98.38 83.17 E 93.316 E 93.310 E 97.71 E	Time Segment: Coceaine Re Calc. Conc. 11 8245 (1) 250768 4 2831 24 5377 24 53777 24 53777 24 53777 24 53777 24 53777 24 53777 24 53777 24 53777 24 537777 24 537777 24 537777 24 537777 24 5377777 24 53777777 24 53777777 24 53777777777777777777777777777777777777	<a implementation="" ins<="" instructure="" td=""><td>Accuracy 433: Merge 925 101.0 100.0 10.</td><td>Int. Me Inspec Probleman Accept Accept Accept Accept Accept</td>	Accuracy 433: Merge 925 101.0 100.0 10.	Int. Me Inspec Probleman Accept Accept Accept Accept Accept
		We Agite Ele Batch I	nt Ma Edit V Table le: * C C C C C C C C C C C C C C C C C C C	ssHunter () () () () () () () () () () () () () (Quantitativ Indiyze Metho Indiyze Batch Samp Type Blank Cal Cal Cal Cal Cal Cal Cal Cal Cal Cal	m ir	Acq. Date-Time 5/12/2006 1:3 PM 5/12/2006 2:0 SPM 5/12/2006 2:0 SPM 5/12/2006 2:0 SPM 5/12/2006 2:0 SPM 5/12/2006 2:0 SPM 5/12/2006 2:1 SP	Test_01	, as well ram for of re Default La Default La Coceine Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	vout	 Cocainut RT 2.433 2.449 2.449 2.445 2.445 2.445 2.445 2.445 2.445 2.445 2.445 	e-d3 Resp. 201 dthe follo 25167/ 199967 9246 48552 24541	S/N M 125 103.61 118.29 98.38 83.17 93.16 97.71 93.30	Time Segment: Cocaine Re Calc. Conc. (1) 11 225 (2) 25 2511 (2) 25	<a <="" p=""> Final Conc. 11 5907 25 2511 125 0768 4 2831 24 5377 4.3735 10 9299	Accuracy 2433: Mergy 92.5 101.0 100.1 85.7 96.2	Int. Me Inspec Problem Accept Accept Accept Accept Accept
		Ha Agito Elle E Batch T T T T T T T T T T T T T T T T T T T	nt Ma Edit V Table le: P C C C C C C C C C C C C C C C C C C C	ssHunter () () () () () () () () () () () () () (Quantitativ lyze Metho analyze Batch mple Type: Samp Cal Cal Cal Cal Cal Cal Cal Cal Cal Cal	m ir	Acq. Date-Time 5/12/2006 1:49 PM 5/12/2006 1:51 PM 5/12/2006 1:51 PM 5/12/2006 1:51 PM 5/12/2006 1:51 PM 5/12/2006 1:51 PM 5/12/2006 2:05 PM 5/12/2006 2:05 PM 5/12/2006 2:05 PM 5/12/2006 2:15 PM 5/12/2006 2:15 PM	nat data, omatogi [p] [△] [△] [△] Rest [] [△] [△] Rest [] [△] [△] Rest [] [△] [△] [△] [△] [△] [△] [△] [△] [△] [, as well ram for of re Default Lan Default Lan Coceine Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	vout	 Cocainu RT 2 433 2 453 2 453 2 455 2 460 2 446 	e-d3 Resp. 20 104 the folloo 251074 90949 19949 19949 19949 2464 48582 24641	S/N M 125 wing proble 03.81 118.29 98.38 83.17 93.16 93.30 93.30	Time Segment: Cocaine Re Calc. Conc. 11 8245 (25 2511) 25 2511 24 5377 4 3735 10 9299	<a <="" p=""> Suits Final Conc. 11.8255 Seek at RT = 2 25.2511 125.0768 4.2527 4.3735 10.9299	Accuracy 2433: Mergy 92.5 101.0 100.1 86.7 98.2	Int. Me Problem Accep Accep Accep
		His Agite Ele E Ele Sampl Image: Sample Image: Sample </td <td>nt Ma dit V Table le: Table C C C C C C C C C C C C C C C C C C C</td> <td>ssHunter (() () () () () () () () () () () () () (</td> <td>Quantitativ alyze Metho mple Type: Samp Type Blank Cal Cal Cal Cal Cal Cal Cal Cal</td> <td>P Analys c Analys d Upda d Upda d Q i Q</td> <td>acssage for th is : DrugsOfAbuse - iiii is : DrugsOfAbuse - iiii is : Baport I cols He Layout: : :::::::::::::::::::::::::::::::::</td> <td>nat data, omatogi [rest_01] [p] [△] [公] Rest [] Cocaine [] Co</td> <td>, as well ram for of are Default Lay Coceine Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS</td> <td>vout</td> <td> B. Cocain RT 2439 2449 2</td> <td>e-d3 Resp. 20 d the folloo 509497 9246 48562 9735 24841</td> <td>S/N M 125 0 103.51 0 118.29 96.33 0 93.31 0 97.71 0 93.30 0</td> <td>Time Segment: Cocaine Re Cele: Conc. 11 10255 m(s) with the p 25 2511 225 2511 225 2511 24 5377 4 3735 10 9299</td> <td><a 0768="" 10="" 11="" 1225="" 125="" 24="" 25="" 2831="" 3735="" 4="" 5377="" 9299<="" conc.="" final="" suits="" td=""><td>Accuracy 433 Merge 925 101.0 100.1 85.7 98.2</td><td>Int. Me Int. Me Problem Accept Accept Accept</td></td>	nt Ma dit V Table le: Table C C C C C C C C C C C C C C C C C C C	ssHunter (() () () () () () () () () () () () () (Quantitativ alyze Metho mple Type: Samp Type Blank Cal Cal Cal Cal Cal Cal Cal Cal	P Analys c Analys d Upda d Upda d Q i Q	acssage for th is : DrugsOfAbuse - iiii is : DrugsOfAbuse - iiii is : Baport I cols He Layout: : :::::::::::::::::::::::::::::::::	nat data, omatogi [rest_01] [p] [△] [公] Rest [] Cocaine [] Co	, as well ram for of are Default Lay Coceine Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	vout	 B. Cocain RT 2439 2449 2	e-d3 Resp. 20 d the folloo 509497 9246 48562 9735 24841	S/N M 125 0 103.51 0 118.29 96.33 0 93.31 0 97.71 0 93.30 0	Time Segment: Cocaine Re Cele: Conc. 11 10255 m(s) with the p 25 2511 225 2511 225 2511 24 5377 4 3735 10 9299	<a 0768="" 10="" 11="" 1225="" 125="" 24="" 25="" 2831="" 3735="" 4="" 5377="" 9299<="" conc.="" final="" suits="" td=""><td>Accuracy 433 Merge 925 101.0 100.1 85.7 98.2</td><td>Int. Me Int. Me Problem Accept Accept Accept</td>	Accuracy 433 Merge 925 101.0 100.1 85.7 98.2	Int. Me Int. Me Problem Accept Accept Accept
		Tris Agillo Image: Image and the image an	nt Ma dit y Table le: C C C C C C C C C C C C C C C C C C	ssHunter [] <	Quantitativ alyze Metho maleze Batch Sample Type Blank Cal Cal Cal Cal Cal Cal Cal Cal Cal Cal	m ir e Analyse d Upda e cAll>	tessage for the second se	nat data, omatogi [rest_01] [p] [△] [2] Rest [1: Cocaine [2: 5000 [2: 5000 [2: 5000 [2: 5000 [2: 5000 [2: 5000 [2: 5000 [2: 5000 [2: 5000] [2: 5000 [2: 5000] [2: 5000] [2: 5000 [2: 5000] [2: 500]	, as well ram for of ore Default Lay Cocoine Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	vout	 Cocain RT 2433 2449 2449 2445 2455 2460 2446 	-d3 Resp. 20 d the following 25/07/ 199967 20049 199967 24841 24841 24841 24841 24841 24841 24841 24841 24841 24841 24841 24841 24841 24841 24841 24852 24841 24852 248552 24852 24852 24852 24852 24852 24852 24852	S/N M 1 25 98.33 [97.71 [93.30] 97.71 [93.30]	Time Segment: Cocaine Re Cale: Conc. 11 8225 m(s) with the p 71 5827 25 2511 25 251 24 5377 4 3735 10 9299	<a td="" •="" •<=""><td>Accuracy 2.433: Merge 92.5 101.0 100.1 85.7 98.2</td><td>Int. Me Int. Me Interpretation Accept Accept Accept</td>	Accuracy 2.433: Merge 92.5 101.0 100.1 85.7 98.2	Int. Me Int. Me Interpretation Accept Accept Accept
		Trig Agille Image: Image and the second s	nt Ma Edit \ Table le: C C C C C C C C C C C C C	ssHunter a a a a a a a a a a a a a a a a a a a	Quantitativ alyze Metho mple Type: Samp Diank Cal Cal Cal Cal Cal Cal Cal Cal	m ir e Analyse d Upda d Upda e e All> c k L1 L2 L3 L4 L4 L5 L2 L4 L4 L4 L4 L4 L4 L4 L4 L4 L4 L4 L4 L4	Hesssage for the segret of the segret character of the segret characte	1: Cocaine 2.5000 1: Cocaine (C Exp. Conc. 2.5000 2.500	, as well ram for of are Default Lay Cocaine Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	vout	e E. Cocaln RT 2 4393 2 449 2 440 2 449 2 br>449 2 449 2 449 2 449 2 449 2 449 2 449 2 449 2 449 2 449 4 449 4 449 4 449 4 449 4	a-d3 Resp. 201 19967 9246 48582 924641 9735 24841	S/N M 1 25 103.87 98.33 93.16 97.71 93.30 94.50 94.50 94.50 94.50 94.50 94.50 94.50 94.50 94.50 94.5	Time Segment: Coccaine Re Cale: Conc. 11 8235 m(s) with the p 17 550768 4 8283 24 5377 4 3735 10 9299	<a <="" p=""> suits Final Conc. 11 8225 ceek at RT = 2 24.5377 4.3735 10 9259	Accuracy 2433: Merge 92'5 101.0 100.1 85.7 98.2	Int. Me Int. Me Interpretation Accept Accept Accept Accept
		Image: A galled Image: A galle	nt Ma Edit \ Table le: * C C C C C C C C C C C C C	ssHunter Jai 2 6 Name Blanksi Jaib-L Sample-2 Jaib-L Active Sample-2 Sample-3 Informati P 2 2 2 2 2 2 2 2 2 2 2 2 2 2	Quantitativ alyze Metho nalyze Batch mple Type: Samp Type Blank Cal Cal Cal Cal Cal Cal Cal Cal	m ir Analys d Upda d Upda d Upda d Upda log i level l2 l3 l4 l5 l2 l4 l l2 l3 l4 l5 l2 l4 l l2 l3 l4 l5 l2 l4 l5 l2 l4 l5 l2 l2 l3 l4 l5 l2 l2 l3 l4 l5 l2 l3 l4 l5 l2 l3 l4 l5 l2 l4 l5 l2 l4 l5 l2 l4 l5 l3 l4 l5 l2 l4 l5	acssage for the segret of the segret of the segret for the segre	1: Cocaine 2: 5000 1: 5000	, as well ram for of ore Default Lay Occaine Methol Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	vout vout	B. Cocain RT 2 439 2 453 2 453 2 453 2 453 2 453 2 455 2 450 2 455 2 450 2 455	a-d3 Resp. 201 199967 9246 48562 9735 24841	S/N M 1 251 13.51 C 98.33 C 93.16 97.71 C 93.30 C	Time Segment: Coccaine Re Calc. Conc. 11 8245 m(s) with the p 11 5807 25 2511 25 20768 4 3735 10 9299	A C D C C C C C C C C C C C C C C C C C	Accuracy 2:433: Merge 92:5 101:0 100.1 105:7 98:2	Int. Me Problem Accept Accept Accept
		Weight Applie File Batch I Sample I	nt Ma alit V Table le: Table le: C C C C C C C C C C C C C C C C C C C	sstiunter (ie and is a second	Quantitativ alyze Metho mple Type: Samp Type Blank Cal Cal Cal Cal Cal Cal Cal Cal Cal Cal	m ir e Analy: d Upda d Upda (All> Level L1 L2 L3 L4 L5 L2 L4 L4 L5 L2 L4 L4 L5 L2 L4 L4 L5 L2 L4 L4 L5 L2 L4 L4 L5 L5 L5 L5 L5 L5 L5 L5 L5 L5	Acq Dete-Time 5/12/2006 1:5 PM 5/12/2006 1:5 PM 5/12/2006 1:5 PM 5/12/2006 1:5 PM 5/12/2006 1:5 PM 5/12/2006 1:5 PM 5/12/2006 2:0 PM 5/12/2006 2:0 PM 5/12/2006 2:1 P	Test_01	, as well ram for of ore Default La Deceme Metho Int. MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS MS-MS	out out Int Parms.	2 2 2 2 2 2 2 4 2 2 4 2 2 4 3 2 4 4 2 4 4 2 4 4 3 2 4 4 4 2 4 4 4 2 4 4 4 5 2 4 4 4 5 2 4 4 4 5 2 4 4 4 5 2 4 4 4 5 2 4 5 2 4 5 2 4 5 2 4 5 2 4 5 2 4 5 2 4 5 2 4 5 5 2 4 5 5 2 4 5 5 5 5 5 5 5 5 5 5 5 5 5	e-d3 Resp. 201 d the follo 251674 199967 9246 445522 924841 248441 248522 248441 248522 248441 248522 248441 248522 248441 248522 248441 248522 248441 248522 248441 248522 248441 248522 248441 248522 248441 248522 248441 248522 24852	S/N M 1 251 115.81 2 118.82 2 98.38 2 93.16 93.17 2 93.10 2	Time Segment: Coceine Re Calc. Conc. 11 8245 (25 2511) 125 0768 4 2831 24 5377 24 5372 24 53772 24 5377 24 5377 24 5377 24 5377 24 5377 25 547 24 5377 24 53777 24 53777 24 537777 24 53777777777777777777777777777777777777	CA To Conc. Final Conc. 11 1825 Seek at RT = 2 24 5377 4.3735 10 9299 10 9299	Accuracy 2433: Mergy 92 5 100.1 85.7 98.2	Int. Me Incomp Accept Accept Accept Accept

Agilent 6410 Triple Quad LC/MS Familiarization Guide

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 2.8 2.9 3 Acquisition Time (min)

Blank-1 Cocaine 11 Samples (11 total) .::

0.4-

2.5-2-1.5-1-0.5-0--0.5-

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 2.8 2.9 3 Acquisition Time (min)

Task 2. Integrate without parameters

Steps	Detaile	d Ins	truct		Com	Comments								
	f Click the F the F the s MDN g Sele the I The mess integ	the Batch Previo Batch Syste /IA. ct the syste sage grate	Next Tabl Dus C Tabl m dis e Bla letric em dis for th d chro	Comp e Stan ompou e Stan plays nk-1 ro colum splays nat dat omato	ou da un da the ow in. ar ar a, gra	and icon ard tool b d icon b ard toolb e compo v, and po v, and po ny outlie as well am for N	in par or in ar until und int to r as the 1DMA.	 Th <i>fo</i>	ne outli MDMA: Ilowing Γ = 2.46 ote tha e integ reen - <i>I</i> lue - In ed - Re nese co e peak	er me Inte prob 64: I 64: I ratio Acce spec jecte lors colo	essag grato blems nterfo se co n met pted t are al are al rs.	jes r fo s wi erer lors tric:	read und th th nce s app	ls the peak at Problem". bear for ected in
 4 Change the noise algorithm. Add the Noise Algorithm column from the Compound Method list. View the values in the Noise Alg. and S/N columns for amphetamine. 	 a Righ Table Colu The dialo b Sele Sele Type and The colu the c d Click e Click the E syste f Exan and S colu 	t-clicc mns. syste g box ct Co ct Co	k any d sele am dis x. mpo lumr ise <i>A</i> n the Add . o the list. Previ a Tabl splay the va (sign	where ect Add splays und M is Froi Ng. (N Availa oves th Show ous Co s the alues i alues i al-to-r	th et noi: ab th ba co noi: ba	n the Ba Remove the Colum hod fron dropdow se Algor le Colum selected the Solution ar an an an an an an an mpound in the Nois ise ratio)	tch ins in the vn list. ithm nns list, d umns in con in ntil the Amp. ee Alg.							
	Amp		-	ISTD:	A	mp-d5	Т	ime Segme	nt: <a th="" 🔻<=""><th></th><th>E 🙀 🗭</th><th>• 🕐 1</th><th><u>ب</u></th><th></th>		E 🙀 🗭	• 🕐 1	<u>ب</u>	
			-			Amp Res	ults			Qualif	fier (119.	.4)	Amp	
	Naine Al-	DT	Deer	C/N	MI	Cala Cara	Final Car -	Acouració	Int Matria	Datia	CIN	M	DT	
	Noise Aig.	RI	rtesp.	SIN	IVII	Calc. Conc.	rinar Conc.	Accuracy	Int. Wetric	Ratio	S/IN		RI	
	RMS	2 141	658	49 10	+	2 4296	2 4206	97.2	Accented	24.3	45.47	H	2 120	
	RMS	2.140	1059	42.25		4.8673	4.8673	97.3	Accepted	33.5	Infinity	Ы	2.12	
	RMS	2.134	2673	107.28		12.8217	12.8217	102.6	Accepted	26.7	146.48		2.12	
	RMS	2.022	4952	20.26		25.9349	25.9349	103.7	Accepted	29.1	49.40	旧	1.990	
	RMS	2.101	1006	47.90		123.9465	123.9465	99.2	Accepted	27.0	39.22	H	2.0/6	
	RMS	2.135	4716	91.48		26.9858	26.9858	107.9	Accepted	25.6	60.79	T	2.12	
	RMS													
	RMS	2.143	1004	80.65		4.0131	4.0131		Accepted	30.9	70.54		2.130	
	RMS	2.105	2590	74.97		13.3607	13.3607		Accepted	25.3	65.40		2.08	

Steps	Detailed Instructions	Comments
 5 Practice changing the noise algorithm from RSM to ASTM for amphetamine in the method. • Exit, but don't save, the method. 	 a Select Method > Edit to switch method editing mode. b Select Method Tasks > Advan Tasks > Integrator Parameters. The system displays the integr parameters in the Method Table 	n to ced s Setup. ator e.
	Method Tasks ×	
	New / Open Method	
	Method Setup Tasks	
	MRM Compound Setup MR Retention Time Setup SISTD Setup Concentration Setup Cualifier Setup Calibration Curve Setup	
	🖉 Globals Setup	
	Save / Exit	
	ừ Validate	
	📾 Save	
	Save As	
	Compound Setup Compound 2D Setup Integration Parameters Setup Smoothing Setup Mass Extraction Setup Isotopic Dilution Setup Browse Acquisition Method C Click the Noise Alg. column for the Method Table. A list of available Noise Algorit appears.	Amp in thms

Steps	Detail	ed Instructi	ons		Comr	nents		
	Int. MS-MS Peak-to Peak-to ASTM RMS	Noise ASTM o-Peak o-Peak from Di	Alg. V					
	e Sele Exit f Clic like bat The mod	ect Method t. ek No to the e to apply th ch? e system dis de.	Tasks > 3 exit prom is method plays Bat	Save/ pt W I to th ch Ar	'Exit > ould you ne nalysis			
 6 Turn the baseline (highest concentration standard) off and then back on for amphetamine. Make sure that only the Compound Information pane is 	a Sela alre Ma icor	ect sample (eady selecte ximize Com n in the tool	Calib-L5 (d), and cl pound In bar.	if it is ick th forma	not • No e for ation the	tice that tl the quant default se	he baselir ifier chroi etting.	ne is drawn in natogram as
visible in the window.	Hi Agil	ent MassHunter	Quantitative	Analys	is - DrugsOfAbuse - iii_	_Test_01		
chromatograms: one with the	Eile	Edit View Ana	alyze <u>M</u> ethod Analyze Batch	Upda	te <u>R</u> eport <u>T</u> ools <u>H</u> el Layout: 拱 🔛 🕅 🕅	p	re <u>D</u> efault La	yout
baseline on and the other with it	Batch	Table						
off.	i Samp	ole: 👔 🌉 🛛 Sa	mple Type: <	All>	👻 🛛 Compound: 🔙 1	L: Cocaine		-
			Samp	е			Cocaine	Metho
	۲	V Name	Туре	Level	Acq. Date-Time	Exp. Conc.	Int.	Int. F
		Blank-1	Blank		5/12/2006 1:48 PM		MS-MS	
		Calib-L1	Cal	12	5/12/2006 1:51 PM	2.5000	MS-MS MS-MS	
		Calib-L2	Cal	13	5/12/2006 1:54 PM	12 5000	MS-MS	
		Calib-L4	Cal	L4	5/12/2006 2:00 PM	25.0000	MS-MS	
	► E	Calib-L5	Cal	L5	5/12/2006 2:03 PM	125.0000	MS-MS	
		QC-L2	QC	L2	5/12/2006 2:06 PM	5.0000	MS-MS	

Steps	Detailed Instructions	Comments						
	b Right-click either of the chromatograms to bring up the shortcut menu.	 Notice that the baseline disappears after deselecting it in the shortcut menu. 						
	 Copy CtrI+C Chromatogram Qualifiers JL Spectrum Spectrum Spectrum Spectrum Auto Scale X - Auto Scale X - Auto Scale Y - Auto Scale Et to Peak Peak Labels Baselines Fill Peaks Baselines Wormalize Qualifiers Uncertainty Band Clear Manual Integration Print CtrI+P Print Preyjew C Clear the Baselines checkbox in the shortcut menu. d Right-click either of the two chromatograms, and mark the Baselines checkbox in the shortcut menu. e Compare the chromatograms with a without a drawn baseline. 	e t and						
	Compound Information Image: State of the st	Compound Information Image: Second second						
	2 5 No baseline 2 - 1.5 - 1 - 0.5 - 0 - 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6 2.8 Acquisiton Time (min	2.5- baseline 2- 1.5- 1- 0.5- 0- 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6 2.8 3 Acquisition Time (min)						

Steps	Detailed Instructions	Comments
7 Inspect the calculation points for the baseline for amphetamine.	 a Right-click either of the two chromatograms, and mark the Baseline Calculation Points checkbox in the shortcut menu. You can now see where the baseline starts and stops. b Right-click either of the two chromatograms, and clear the Baseline Calculation Points checkbox in the shortcut menu. c Compare the chromatograms with and without Baseline Calculation Points. 	
	Compound Information • MRM (136.2 -> 91.4) CMAMCal_L5.d • MRM (136.2 -> 91.4) CMAMCal_L5.d • 5 •	Compound Information

Task 2. Integrate without parameters

Steps			etailed Instructions	Comments	
8	 Display the peak labels for amphetamine. Display those found in the figure on the next page. Then display the original retention time peak label. 	a b	Right-click either of the two chromatograms, and select Peak Labels from the shortcut menu. The system displays the Compound Information dialog box. Mark all the Peak Labels check boxes, and the Display Label Names check box, and click OK .		

Compound Information	? 🛛
Peak Labels ✓ RT ✓ Name ✓ Calc. Conc. ✓ Height ✓ Area	Move Up Move Down
Display Label Names (ex. RT=2.452 OK Cancel]] Apply

The peak labels should now match those shown in the example below.

Compou	Compound Information ×								
	■ ■ 📐 五 业 🛃 🖬 🕶 💠 🏠								
+ MRM (13	6.2 -> 91.4) CMAMCal_L5.d								
2 ×10 3	RT=2.101								
ତି 4-	Calc. Conc. = 123.9465 Height=3807.3455								
3.5-	Area= 10005.3105								
3-									
2.5-									
2-									
1.5-									
1-									
0.5-									
0-									
	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 2.8 2.9 3 Acquisition Time (min)								

Steps	Detailed Instructions	Comments					
	 c Right-click either of the two chromatograms, and select Peak Labels from the shortcut menu. The system displays the Compound Information dialog box. d Clear all the Peak Labels checkboxes except RT (retention time). Clear the Display Label Names checkbox, and click OK. 						
9 Display the qualifier chromatogram before and after normalization.	 a Right-click either of the two chromatograms, and mark the Normalize Qualifiers checkbox in the shortcut menu. The two peaks now converge and appear as one peak. b Right-click in the Compound Information window, and clear the Normalize Qualifiers checkbox in the shortcut menu. c Compare the qualifier chromatogram with and without normalization. 	 Notice that the default setting displays the qualifier peak overlaid on the quantifier peak before normalization. 					
	136.2 -> 91.4 . 136.2 -> 119.4 \$	136 2 -> 91.4 . 136 2 -> 119.4 (2) x10 2 12 12 14 15 12 12 14 16 18 18 18 18 18 18 18 18 18 18					

Steps	Detailed Instructions	Comments					
10 View the uncertainty band.	 a Right-click either of the two chromatograms, and mark the Uncertainty Band check box in the shortcut menu. The uncertainty band appears in the qualifier chromatogram. b Right-click either of the two chromatograms, and clear the Uncertainty Band checkbox in the shortcut menu. c Compare the qualifier chromatogram with and without Uncertainty Band. 	 uncertainty band - a dashed band that shows the upper and lower boundaries for the qualifier abundance 					
11 Remove the Int. and Int. Parms.	a Right-click the Batch Table, and selec	136.2 > 91.4 . 136.2 > 119.4 g x10 ³ Ratio=27.0 3.5 2.5 2.5 2.5 1.5 1.5 1.2 1.4 1.6 1.8 2.2 2.4 2.6 2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5					
11 Kemove the Int. and Int. Parms. columns.	 a Right-click the Batch lable, and selec Add/Remove Columns. b Select Int. and Int. Parms. (Compound Methods) from the right-hand list. c Click Remove, then OK. 	t					

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.

Steps			iled	Instru	ictions			Comn	nents						
1 View outlier information for MDMA.	a b	CI Ta di Se cu th	ick I ble spla elect irsor e ex	Vext (toolba ys the the E to th ample	Compou ar Ii IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	ind in til the bund l row, lumn	the Batch system MDMA. and point the , as shown in								
	i i	Batch Table ×													
		Sam	ole: 👰	J Sa	mple Type	<a td="" 👻<=""><td>Compound: 🔄 1. 💌</td><td>ISTD: M</td><td>M</td><td>Seament</td><td><a td="" 🗸<=""><td>11: CX</td><td></td><td>7</td></td>	Compound: 🔄 1. 💌	ISTD: M	M	Seament	<a td="" 🗸<=""><td>11: CX</td><td></td><td>7</td>	11: CX		7	
	-	Jaili		<u>v</u>] 38	Sam	ple	compound. (e) 1. Y	MDMA	Method			E: 44	~ (MDM	
		•	\$	Name	Туре	Level	Acq. Date-Time	Exp. Conc.	Noise Alg.	RT	Resp.	S/N	мі	Calc. (
	•	0	K E	Blank-1	Blank		5/12/2006 1:48 PM		RMS	2.466	26	2.37		6	
		-	2	alib-L1	Cal	L1	5/12/2006 1 Y Outli	er(s)						_	
		Calib-L2 Cal L2 5/12/2006 1 MDMA: Retention time = 2.466 is o									utside the allowed range [2.158, 2.385]				
			C	Calib-L3	Cal	L3	5/12/2006 1:57 PM	12.5000	RMS	2.277	17023	118.22		11	
		_	0	alib-L4	Cal	14	5/12/2006 2:00 PM	25.0000	RMS	2.264	33212	93.82		24	
		-	0	alib-L5	Cal	L5	5/12/2006 2:03 PM	125.0000	RMS	2.271	110142	126.31		125	
		-	(2C-L2	QC	L2	5/12/2006 2:06 PM	5.0000	RMS	2.2/6	/253	88.86	님	4	
	-	0		C-L4	QC	L4	5/12/2006 2:09 PM	25.0000	RMS	2.2/6	31464	105.63	님	23	
			T 3	Sample-1	Sample		5/12/2006 2:12 PM	-	RIVIS	2.315	4/0	106.67	님	5	
		-	2	ample-2	Sample	-	5/12/2006 2:15 PM		DMC	2.200	16710	116.02	片	10	
	1			ample-5	Sample		5/12/2000 2. 10 F W		TRIVI3	2.207	10/10	110.02		10	
		<u>د</u>			111									2	
	10	Comp	ound	nformati	ion									×	
	1	1			金山会。	. : 2	↔ ‡ ⚠								
	+	MRM	(194.2	-> 163.2)	CMAMBIk_0)1.d		194.2 -> 163.2	194.2 -> 13	35.3					
	st					1	2.486557	≌ , Ratio	=30.2						
	our	2				1		4-				ЛЛ			
	0	3.	2		1 776	2.28	1 0 586	3.5-				1111			
			3-		1.770	2.20	T HIDOO	3-			п				
		2.	5-			<mark> </mark>		25-			- 11	(1)			
			>					20							
		1						2-				FIII			
		1.3	2					1.5-			11 11				
			-		1			1-			lth nt 1	H H		-	
		0.	5-					0.5-				++++			
		(_				0			111				
														(****)	
			1.4	4 1.6	1.8 2	2.2	2.4 2.6 2.8	1.4	1.6 1.	8 2	2.2	2.4 2.1	5_2	.8	

Task 3. Detect outliers

ps Detailed Instructi								uction	IS			C	ommei	ıts			
					C	: Exam Quali Samp belov	ine th fier Ile 1, a v.	e outl Resul is sho	ier ts wr	r informa > Ratio n in the e	tion in t column example	he for					
Agile	nt /	MassHunter	Quantitative	e Analys	sis - DrugsOfAbuse	- iii_Test_0	1										
ile I	Edit	View An	alyze <u>M</u> etho	d <u>U</u> pda	te <u>R</u> eport <u>T</u> ools	Help											
			Analyze Batch	10:	Lavout:		Restore I	Default La	vou	t							
atab	Tal	hle						7	,								
atch	Tai	Die							-						_		
Samp	le:	🚹 🐌 Sa	mple Type:	<all></all>	Compound:	🛥 1: MDM/	4		- [ISTD: MD	MA-d5	1	Time Segme	nt: <a< td=""><td>-</td><td>•</td><td>100</td></a<>	-	•	100
			Samp	le						MDMA Res	ults			Qualif	ier (135.3)	MDM	A-d5 (I
~	LANS N	1		1													
D	P	Name	Туре	Level	Acq. Date-Tim	e RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Int. Metric	Ratio	S/N M	AI RT	Resp.
0	*	Blank-1	Blank	1	5/12/2006 1:48 PN	2.466	26	2.37		6.9724	6.9724		Rejected	30.2	0.94	2.602	2 28
		Calib-L1	Cal	L1	5/12/2006 1:51 PM	2.276	3794	97.50		2.2824	2.2824	91.3	Accepted	10.2	41.49	2.275	5 12175
		Calib-L2	Cal	L2	5/12/2006 1:54 PN	2.277	7433	94.47		4.6561	4.6561	93.1	Accepted	11.0	58.15	2.27	5 11691
		Calib-L3	Cal	L3	5/12/2006 1:57 PM	2.277	17023	118.22		11.2728	11.2728	90.2	Accepted	10.0	83.01	2.276	5 11059
		Calib-L4	Cal	L4	5/12/2006 2:00 PM	2.264	33212	93.82		24.8702	24.8702	99.5	Accepted	9.6	101.60	2.262	2 9780
		Calib-L5	Cal	L5	5/12/2006 2:03 PN	2.271	110142	126.31		125.1668	125.1668	100.1	Accepted	9.6	95.77	2.268	8 6444
		QC-L2	QC	L2	5/12/2006 2:06 PM	2.276	7253	88.86		4.8567	4.8567	97.1	Accepted	9.6	48.25	2.274	4 10938
		QC-L4	QC	L4	5/12/2006 2:09 PM	2.276	31464	105.63		23.0331	23.0331	92.1	Accepted	9.1	107.02	2.274	4 10004
0		Sample-1	Sample	-	5/12/2006 2:12 PN	2.315	476	11.26		5.6138	5.6138		Accepted	12.6	5.39	2.314	4 620
		Sample-2	Sample		5/12/2006 2:15 PM	2.280	7651	126.67		5.1778	Y Ou	tlier(s)					
		Sample-3	Sample		5/12/2006 2:18 PN	2.267	16710	116.02		10.7772	1 MDMA	Qualifier r	atio = 12.6 is	s outside	the allow	ed range	7.6, 11.5]
omp	oun	d Informati	ion					×	Са	libration Cur	ve						×
3			🔣 山 🔬 📕	2	↔ ‡ <u><u>R</u></u>					📄 Туре:	Linear	 Origi 	n: Force	- W	eight: Non	e 🕶 <u>I</u> !	STD QC
IRM I	(194	.2 -> 163.2)	CMAMSam_	01.d	194.2 -> 163.2	194 2 -> 135	3		7	↔ ± 3% -							
x10 ²			2.315		월 x10 ² Ratio=	12.6		6			5 Lovola Llaor	E Dointe	5 Pointe Llev	4 2 0 0	c		
	1		1		3 1.6		1	II'	0	10.1 y = 6.82	73 * v	I, S FOILIS,	J Fullits Use	5u, 2 QC	5		
1.4	-				1.4-				asr.	$R^2 = 0$	99984076						×
1.0							1		ode	1.6-						/	1
1.2	1				1.2-				Het	1.4-					-	/	
1	+				1		1		Ne	1.2-					/		
									ati	1				/			
0.8	1				0.8-		1111	6	Re				/				
0.6	-				0.6-					0.8-			/				
0.0					0.4					0.6-		/	The second				
0.0	-				0.4-					0.4-		/					
0.4																	
0.4	-				0.2-		۵			0.2-							
0.4	1	mn	wolla	m	1 0.2- 1 0	and	the	ma		0.2-	· · ·						

- 2 Change the accuracy range for amphetamine in the method, and re-analyze the batch.
 - Set the accuracy maximum percent deviation (Accuracy Max % Dev): to 5%.
- a Click the **Previous Compound** icon in the toolbar until the system displays the compound **Amp**.
- **b** Select the **Calib-L5** row in the table.
- c Select Method > Edit to switch to method editing mode.
- d Select Method Tasks > Outlier Setup Tasks > Accuracy.
- e 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.

Commente

Task 3. Detect outliers

Agilent MassHunter Quantitati	ve Analysi	is - [I	lew Method]					
<u>File Edit View Analyze Meth</u>	od <u>U</u> pdal	te <u>R</u>	eport <u>T</u> ools <u>H</u> elp					
🛅 🗁 🛃 🖬 🕻 🗊 Analyze Bate	h 🕘 🗄	Layo	ut: 🔜 🔢 🕅 🛄 🔼	🔀 Rest	ore <u>D</u> efault Layout			
Method Tasks	×	Me	thod Table					×
Method Setup Tacks	^	Т	ime Segment: 👄 <a< td=""><td>. ></td><td>👻 📫 🗍 Compound: </td><td>🖛 Amp-d5 🛛 💌 🛤</td><td><u>R</u>eset Table View</td><td></td></a<>	. >	👻 📫 🗍 Compound:	🖛 Amp-d5 🛛 💌 🛤	<u>R</u> eset Table View	
месной эссир тазкэ	_	1	Sample					
MRM Compound Setup			Name	_	Aca Date-Time	Data File		
C Retention Time Setup		2-	Calib-L5		5/12/2006 2:03 PM	CMAMCal 15 d		
😰 ISTD Setup		-	0000 200	_	0/12/2000 2:001 111	_ on a modi_co.d	1	
Concentration Setup			Quantifier	_	7			
🥂 Qualifier Setup	=		Name	on	Scan	Туре	Accuracy Max. % Dev.	LOQ Accuracy Multiplier
Calibration Curve Setup			Amp	4	MRM	Target	5.0	1.0
Clabala Satura	_		Amp-d5	4	MRM	ISTD	20.0	1.0
Globals Setup	_		Cocaine	2.0	MRM	Target	20.0	1.0
Save / Exit			Cocaine-d3	5.0	MRM	ISTD	20.0	1.0
2 Validata	_		MDMA	8.2	MRM	larget	20.0	1.0
a validate			MDMA-d5	+.3	MRM	ISID	20.0	1.0
Save			Meth-d5	- 2.5	MDM	Isto	20.0	1.0
Save As			Wether		WPW	1010	20.0	1.0
X Exit								
Manual Setup Tasks								
Outlier Setup Tasks				Tv	o different scr	oll bars are ava	ailable to allow y	ou to
Accuracy				m	ove the two sec	tions separate	ly.	
7 Qualifier Ratio	_					-		
A Retention Time				_				
and internation time								

Detailed Instructions

Stone

- f Select Method Tasks > Exit, and click Yes in the confirmation prompt, to exit the method and apply the method to the batch.
- g 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.

				Sampl	F	Results								
۲		Ÿ	Name	Туре		Final Conc.	Accuracy	Int. Metr						
	0		Blank-1	Blank										
			Calib-L1	Cal	6	2.4296	97.2	Accepter						
		٣	Calib-L2	Cal	3	4.8673	97.3	Accepter						
			Calib-L3	Cal	7	12.8217	102.6	Accepter						
			Calib-L4 Cal		9	25.9349	103.7	Accepter						
			Calib-L5	Cal	5	123.9465	99.2	Accepter						
		٣	QC-L2	QC	7	4.3457	86.9	Accepter						
		۴	QC-L4	QC	β	26.9858	107.9	Accepter						
۲	•		Sample-1	Sample										
			Sample-2	Sample	1	4.0131		Accepter						
			Sample-3	Sample	7	13.3607		Accepter						
				>	:									

Task 3. Detect outliers

St	eps	De	etailed Instructions	Comments				
3	Using the following set of outlier flag icons X Y Y Y Y Y : • Check for samples with high outliers		Click the Display samples that have High outliers icon on the toolbar to display only samples with high outliers.	 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 				
	 Check for samples with both high and low outliers 	b	Click the Display samples that have		outliers.			
	 Display all samples again. 		toolbar to display only samples with					
	Hide the outlier flags for		low outliers.					
	Accuracy and RT for Amp.	C	Click the Display samples that have					
	 Show these outlier flags again 		High/Low outliers realized icon again to display all the samples.					
		d	Click the Select Outliers 📢 icon to					
			bring up the Outliers dialog box.					
		е	Clear the Accuracy and Retention					
			Time check boxes, and click OK .					
		f	Click the Select Outliers 📢 icon to					
			bring up the Outliers dialog box.					
		g	Mark the Accuracy and Retention					
			Time check boxes, and click OK .					



Agilent 6410 Triple Quad LC/MS Familiarization Guide

Exercise 5 Work with quantitation reports

Task 1. Generate quantitation reports94Task 2. Review the reports99Task 3. Customize a report template101

This exercise helps you learn how to do these tasks:

- Generate reports using specified templates
- Review the reports, looking for outliers
- Customize reports by adding columns and changing the report header

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.



Task 1. Generate quantitation reports

In this task, you generate ISTD and Qualifier Ratio reports using the corresponding templates.

Steps			etailed Instructions	Comments		
1	If necessary, open the batch file <i>iii</i> _ Test_01.batch.xml . If the batch is already open, skip to step 2.	a b c	To start the Quantitative Analysis program, click the Quantitative Analysis icon on your Desktop. Click Open Batch on the toolbar to display the Open Batch dialog box. Navigate to \ <i>Your Directory</i> \ DrugsOfAbuse and select <i>iii_</i> Test_01.batch.xml .	•	You can also access the program by selecting Programs > Agilent > MassHunter Workstation > Quantitative Analysis from the Start menu. If the default layout is not present, click Restore Default Layout on the toolbar before opening the batch. <u>Restore Default Layout</u>	
2	 Verify the default destination directory for reports. The destination directory should be \ Your Directory \ DrugsofAbuse \ QuantReports. The default filename is <i>iii_</i>Test_01, where "<i>iii</i>" are your initials. 	a b	Select Report > Generate . The system displays the Report dialog box. Specify the default destination directory for saving Excel reports in the Report Folder text box; for example, \ <i>Your Directory</i> \DrugsOfAbuse\ QuantReports\ <i>iii</i> _Test_01.	You can also specify the Instrument Type determining numeric formats in graphics files . This value is used to determine how many decimal places to show in the graphics.		

Task 1. Generate quantitation reports

Steps	Detailed Instructions	Comments
	Report Graphics Files: Generate Graphics Files Batch Folder: C\QuantData\DrugsOfAbuse\ Bgtch File: Iii_Test_01.batch.xml Use Existing Graphics Files Graphics Settings: Load Graphics Settings File Instrument Type determining numeric formats in graph Report Eolder C\QuantData\DrugsOfAbuse\QuantReports\tiltit_Test_01-0 Reports: Template	Printer
	New Delete Localize	e: <none></none>

- **3** Add an ISTD template.
 - Add the template, Quantreport_ISTD_B_01_03.xlt.
 - Make sure the report name is *TemplateName*.xls, where *TemplateName* is the exact name of the template.
- a Click New in the Report dialog box. The system displays the Open dialog box.
- **b** Select
 - Quantreport_ISTD_B_01_03.xlt and click Open.

The program adds the template to the Template field in the Reports pane.

- c In the Report File Name field in the Reports pane, verify that the report file name is
 QuantReport_ISTD_B_01_03.xls.
- Note that the B_01_03 designation corresponds to the Quantitative Analysis software release, which will change over time. Therefore, the default report file name may change correspondingly.

Task 1. Generate quantitation reports

Steps	Detailed Instructions	Comments
	Report	
	Keport Graghics Files: Image: Second	≥OfAbuse\ al
	C:\MassHu\QuantReport_ISTD_B_01_03	3 XLT QuantReport_ISTD_B_01_03 xls KNone> Localize: <none> filled in automaticallay. OK Cancel</none>
 Add a Qualifier Ratio template. Add the template, Quantreport_Outlier_Qualifier ratio_B_01_03.xlt. Make sure the report name is <i>TemplateName</i>.xls, where <i>TemplateName</i> is the exact name of the template. 	 a Click New in the Report dial The system displays the Op box. b Switch to the Outliers direc c Select Quantreport_Outlier ratio_B_01_03.xlt and click d In the Report File Name field Reports pane, verify that the name is Quantreport_Outlier_Quali Ratio_B_01_03.xls. 	log box. en dialog tory. •_Qualifier : Open. d in the ∋ report file

Task 1. Generate quantitation reports

Steps	Detailed Instructions	Comments
	Report Graphics Files: @ Generate Graphics Files Batch Folder: C:\QuantData\DrugsOf Bytch File: iii_Test_01batchxml Use Existing Graphics Files Graphics Settings: Load Graphics Settings File Instrument Type determining numeric formats Report Eolder C:\QuantData\DrugsOfAbuse\QuantReports\iii_T Reports: Template C:\MassHunter\Report_ISTD_B_01_03_X	Abuse\ Abuse\ s in graphics files: QQ ▼ Report File Name ▼ Report File Name Printer LT QuantReport_ISTD_B_01_03.xls <none> LT QuantReport_Outlier_QualifierRatio_B_</none>
5 Generate the reports.	a Click OK in the Report dialog b generate the report	Localize: <none> V OK Cancel</none>
generation in the Task Queue Viewer.	 b Select Report > Queue Viewe monitor the report generation The system displays the Task 	er to process. Queue

Viewer dialog box. **c** Watch the progress of the report in the Status column.

Agilent 6410 Triple Quad LC/MS Familiarization Guide

Task 1. Generate quantitation reports

Detailed Instructions		Comments				
Agilent MassHunter Quantitative Ana	lysis - Task Queue Viewer					
File Service Tasks Help						
i 🕟 💷 🗷 🗙 🔍						
Name	Creation Time	Status	Completion	^		
DrugsOfAbuse iii Test 01.20070702.0	95 7/2/2007 9:57:45 AM	Done	7/2/2007 10:00:22 AM			
DrugsOfAbuse_iii_Test_01.20070706.1	04 7/6/2007 10:42:01 AM	Processing		~		
				Connected		
		_		connected ,		
🗿 Agilent MassHunter Quantitative Ana	lysis - Task Queue Viewer					
File Service Tasks Help						
i 🕟 🖬 🖉 🔀 🖻						
Name	Creation Time	Status	Completion	^		
DrugsOfAbuse_iii_Test_01.20070706.1	04 7/6/2007 10:42:01 AM	Done 🗸	7/6/2007 10:45:10 AM			
DrugsOfAbuse_iii_Test_01.20070702.0	095 7/2/2007 9:57:45 AM	Done	7/2/2007 10:00:22 AM	~		
				Connected		
	Detailed Instructions Agilent Massifunter Quantitative Anal File Service Tasks Help OrugoOfAbuse iii Test 01 20070702 (DrugoOfAbuse iii Test 01 20070706 1 File Service Tasks Help OrugoOfAbuse iii Test 01 20070706 1 Name Name OrugoOfAbuse iii Test 01 20070706 1 OrugoOfAbuse iii Test 01 20070706 1 OrugoOfAbuse iii Test 01 20070706 1	Detailed Instructions Agilent MassHunter Quantitative Analysis - Task Queue Viewer File Service Tasks Help Mame Creation Time DrugsOfAbuse iii Test 01 20070702 095 7/2/2007 9:5745 AM DrugsOfAbuse iii Test 01 20070706 104 7/6/2007 10:42:01 AM MassHunter Quantitative Analysis - Task Queue Viewer File Service Tasks Help Service Tasks Help	Detailed Instructions Commen * Aglient MassHunter Quantitative Analysis - Task Queue Viewer File File Service Tasks Help Image: Ima	Detailed Instructions Comments Aglient MassHunter Quantitative Analysis - Task Queue Viewer Fie Service Tasks Help OnucsOtAbuse. iii. Test. 01 20070702 095. ParugsOtAbuse. iii. Test. 01 20070702 095. ParugsOtAbuse. iii. Test. 01 20070705 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070706 104. ParugsOtAbuse. iii. Test. 01 20070702 095. ParugsOtAbuse. iii. Test. 01 20070702 095. ParugsOtAbuse. iiii. Test. 01 20070702 095.		

d Close the Task Queue Viewer.

Task 2. Review the reports

In this task, you review the reports from the last task in Microsoft Excel and look for outliers.

S	teps	D	etailed Instructions	Comments
1	Review the ISTD report generated in the previous task to familiarize yourself with its organization.	a	Go to the directory C:\ <i>Your Directory</i> \ DrugsOfAbuse\QuantReports\ Test_01.	
	 View the organization of each worksheet. 	b	Right-click Quantreport_ISTD_B_01_03.xls, and click Open.	
		C	Inspect the contents of each worksheet in the Excel file.	

M	icrosoft Exc	el - Qua	ntRepo	ort_ISTD_	B_01_0	3.xls													
: 🗐	<u>F</u> ile <u>E</u> dit	View	Insert	F <u>o</u> rmat	Tools	<u>D</u> ata	Window	Report	Design	<u>H</u> elp	Ado <u>b</u> e	PDF				Туре	a que	stion for help	×
: 0	💕 🖬	2.0	8	3 29	2 і т	ahoma		v 10	- 1	BI	U	E E	=	\$	%,	€.0 .00 0.€ 00.	緸	🚝 🖽 🗸 💩	- A -
	Process Re	ort	Clear I	Results	Add	Data 🗭	Add Gran	hics	Add For	matting	SA Ad	vanced	Pronerties		/alidate D	esian 🙃	(in)		
• 11	Δ1		- Cicul I	e .	Batch	Info	ride erop	iner ile		matting	74 110	rancea	Tepercies	-	runduce e	congin 😦	-	7	
_				/×	Daten			MA NI	0 1		D	T	11 1/	14/	VV	7 00			
0	AB		E	FG	H I	J	K L	MIN	0	- Q	RS		UV	VV	XY	ZAA	AB	AC AD AE A	AF AG
8	Sequen	ceiac	bie																
10	Data File					Samp	e Name				Posi	tion	Vo	lume	Level	Sample T	ype	Acq Method File	
11	CMAMBIk_0	1.d				Blank-	1				P1-	C1	5	.0000		Blank		APCIautotune.m	
12	CMAMCal_I	1.d				Calib-L	.1				P1-	C6	5	.0000	L1	Calibration	1	APCIautotune.m	
13	CMAMCal_I	2.d				Calib-L	.2				P1-0	C10	5	.0000	L2	Calibration	n	APCIautotune.m	
14	CMAMCal_I	3.d				Calib-L	.3				P1-0	C11	5	.0000	L3	Calibration	ı	APCIautotune.m	12
15	CMAMCal_I	4.d				Calib-L	4				P1-0	C14	5	.0000	L4	Calibration	1	APCIautotune.m	
16	CMAMCal_I	5.d				Calib-L	.5				P1-0	C17	5	.0000	L5	Calibration	1	APCIautotune.m	
17	CMAMQC_L	2.d				QC-L2					P1-	C9	5	.0000	L2	QC		APCIautotune.m	
18	CMAMQC_L	4.d				QC-L4					P1-0	C15	5	.0000	L4	QC		APCIautotune.m	
19	CMAMSam	01.d				Sampl	e-1				P1-0	C22	5	.0000		Sample		APCIautotune.m	
20	CMAMSam	02.d				Sampl	e-2				P1-	C8	5	.0000		Sample		APCIautotune.m	
21	CMAMSam	03.d				Sampl	e-3				P1-0	C12	5	.0000		Sample		APCIautotune.m	
22																			
23	Quantit	ation	Resu	lts															
25	Target C	ompou	nd	Coca	aine														
27	Data File		(ompound	d	I	STD				Exp Cor	nc I	Response	I	STD Resp	Resp F	atio	Final Conc	Accui
28	CMAMBIk_0	1.d	(Cocaine		C	ocaine-d3						20		15		1.3	11.8235	
29	CMAMCal_I	1.d	C	Cocaine		C	ocaine-d3				2.50	00	5189		20245		0.3	2.3087	
30	CMAMCal_I	2.d	(Cocaine		C	ocaine-d3				5.00	00	9716		20506		0.5	4.2682	
31	CMAMCal_I	3.d	C	Cocaine		C	ocaine-d3				12.50	00	25187		19625		1.3	11.5607	
32	CMAMCal_I	4.d	(Cocaine		C	ocaine-d3				25.000	00	50649		18068		2.8	25.2511	1
33	CMAMCal I	5.d	(Cocaine		C	ocaine-d3				125.00	00	199967		14401		13.9	125.0768	1 🕶
H -	I F H S	ummar	y / (Calibration	1 / C	MAMBIK	_01_d /	CMAM	Cal_L1	_d /	CM/ <								>
Rea	tv																	NUM SCRI	

Task 2. Review the reports

Steps	Detailed Instructions	Comments
 Review the qualifier ratios in the Qualifier Ratio report, Quantreport_Outlier_Qualifier ratio_B_01_03.xls. 	 a Go to C:\Quantdata\DrugsOfAbuse\QuantReports\Test_01. b Right-click<quantreport_outlier_qualifier Ratio_B_01_03.xls, and select Open.</quantreport_outlier_qualifier c Examine the qualifier ratio outliers reported in the Excel file. 	Only Qualifier Ratios that are either High or Low are included in the report.
Microsoft Excel - QuantReport_Outlier_QualifierRatio_B_0	1_03.xls	
Eile Edit View Insert Format Tools Data Wind	ow <u>R</u> eport Design <u>H</u> elp Ado <u>b</u> e PDF	Type a question for help 🔤 🗕 🗗 🗙
🗄 🗋 🔀 🔒 🖂 🎿 🖏 🦈 🍟 Tahoma	•10 • B I U 📑 🗐 😫 💲 % ,	않 🔐 (孝 律) 🖽 🕶 💁 🗕 🗕 🗸 🗸
Process Report 🚺 Clear Results 🐂 Add Data 🗣 Add C	Graphics 👫 Add Formatting 🛠 Advanced Properties 📳 Validate De	sign 🚯 @
A1 👻 🏂		
A B C D E F G H I J K L	M N O P Q R S T U V W X Y	Z AA AB AC AD AE AF AG
9 Qualifier Ratio Outlier Summary Table		
11 Data File Sample Type Compound	Transition Unc Rel/Abs Min	Max Qual Ratio Outlier
12 CMAMCal_L2.d Calibration Amp	136.2 -> 91.4 20.0 Relative 21.2	31.8 33.5 High
14 CMAMSam_01.d Sample MDMA-d5	199.2 -> 164.3 20.0 Relative 18.0	26.9 27.5 High
15 CMAMSam_01.d Sample Meth	150.1 -> 119.3 20.0 Relative 76.7	115.1 59.8 Low
17 Qualifier Ratio Outlier Chromatograms		
19 Data File Sample Type Compound	Transition Unc Rel/Abs Min	Max Qual Ratio Outlier
20 CMAMCal_L2.d Calibration Amp	136.2 -> 119.4 20.0 Relative 21.2	31.8 33.5 High
21 + MRM (136.2 -> 91.4) CMAMCal_L2.d # x10 2 RT=2.140 Name=Amp Calc. Conc.=4.8673	+ MRM (136.2 \rightarrow 119.4) CMAMCal_L2.d $g \times 10^{-2}$ RT=2.145 1.4 Name Calc. Conc. = 1.2 Height=96.0000 1.4 Area=354.5500 0.8 0.6 0.4 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2	4 , 136.2 -> 119.4 2 14 16 18 2 22 24 26 28 (Acquisition Time (min)
24 Data File Sample Type Compound 25 CMAMSam 01.d Sample MDM4	Transition Unc Rel/Abs Min 194.2 -> 135.3 20.0 Relative 7.6	Max Qual Ratio Outlier
+ MRM (194.2 -> 163.2) CMAMSam 01.d	+ MRM (194.2 -> 135.3) CMAMSam 01.d	22 194 2 > 125 3
H + + H Outlier_QualifierRatio	Initial [104.2 > 100.0] ONLY MODELLOT [104.2 > 10.	× 101.2 × 100.0
Ready		NUM SCRL

Task 3. Customize a report template

This task shows you how to:

- add a logo to a report header of the ISTD template
- add a column and change the font color
- · generate and review the new report based on the customized template

Steps	Detailed Instructions	Comments			
 Modify the ISTD report template header. Open Quantreport_ISTD_B_01_03.xlt. Add the ASMS2006logo.bmp file to the header. Look at a preview of the report. 	 a Go to the folder \Report Templates \Quant. b Right-click Quantreport_ISTD_B_01_03.xlt, and select Open from the shortcut menu. c Select View >Header and Footer in the Excel window. The system displays the Page Setup dialog box. d Click the Header/Footer tab. 	 You must open the Excel file in this way (right-click the file name, and select Open) to access an editable file. 			

Quant Summary Report (ISTD)	Print
81	Print Preview
e <u>a</u> der: &[Picture], Quant Summary Report (ISTD)	Options
<u>C</u> ustom Header]
ooter: QuantReport_ISTD_B_01_03.XLT, &[Picture]□Page 1 of ?, Printed at: ✔	
	7

e Click Custom Header.

The system opens the Header dialog box.

Steps	Detailed Instructions	Comments
	 f Move mouse cursor to the Right section, and click the Insert Picture icon. g If you are asked, click Replace on the message asking whether or not to replace the existing picture. 	 The image Agilent_Logo.tif is in the directory Report Templates\Quant\Logo+Header+Footer. You can use this image to learn how to include additional graphics in the header.
	Header	
	To format text: select the text, then choose the font button. To insert a page number, date, time, file path, filename, or tab name: p Insertion point in the edit box, then choose the appropriate button. To insert picture: press the Insert Picture button. To format your picture cursor in the edit box and press the Format Picture button.	osition the OK Cancel
	A Ø G G	
	Left section: Quant Su Report	Immary (ISTD)
	h In the Insert Picture dialog box, select Agilent_Logo.tif and click Insert. You may also need to format the picture by clicking the Format Picture icon. In the Agilent templates, the Height is scaled to 80%.	



- i Click **OK** in the Header dialog box.
- j Click **OK** in the Page Setup dialog box.

Steps	Detailed Instructions	Comments
	k Click the Print Preview icon to v the position of the logo on the pa	 When adding your own company age. logo, make sure that it is an appropriate size to fit in the header.
	Microsoft Excel - QuantReport_ISTD_Custom_01.XLT	
	Next Previous Zoom Print Setup Margins Page Break Preview Close Help	

		Qua	nt Summary R	eport	(ISTD)		24	Aguent lectrolog
Batch Info								
Batch Data Path Analysis Time Report Time Last Calib Update	e		Analyst Name Reporter Name Batch State					
Sequence Table	e							
SampleID	Data File	Sample Name	Position	Volume	Level	Sample Type	Acq Method File	
Quantitation Re	esults							
CMD:Repeat SampleID	CompoundID CompoundID	ISTDCompoundID	Formula Name ISTD Compound					
SampleID	CompoundID	ISTDCompoundID	PrimaryHitPeakID PeakII	0	Data File	Compound	ISTD	Exp Conc
CMD:EndRepeat	CompoundID							

- I Verify the display of the modified header, and click **Close**.
- If more than one design tab is part of the report, you need to make the change on each tab. For this report, you need to change the header on the Design-Summary tab, the Design-Calibration tab and the Design-Sample tab.

Steps	Detailed Instructions	Comments		
 Add a Dilution column. Use QuantitationDataSet_ Map1to find the Dilution column. Change the font color of this column name to Automatic. 	 a Select Data > XML > XML Source. The system displays the XML Source window on the right side of the Excel window. b Click on two different columns in the table that you are adding to. c Drag the element Dilution from the XML Source window, and drop it in the Sequence Table as shown in the example below. 	 See the Online Help for definitions of each of the Quantitation DataSet_Map1 columns that you can add to the report. You can use any of the XML maps that start QuantitationDataSet_Map. If the cell you dragged the element to displays in red, it is because it is because it is not from the same section of the XML Source map as the table's element. 		
	03.XLT			
	S Data Window Report Design Help Adobe PDF			
	Data Add Graphics Add Formatting Advanced Properti	es 🍣 Validate Design 🚯 🖗		
	ion			
	G H I	∧ XML Source ▼ ×		
		000		
	First, click on two different fields	XML maps in this workbook: QuantitationDataSet_Map1		
	in the table.			
		ns1:AcgMethodHeName		
		- Ins1:AcqMethodPathName		
		s1:BalanceOverride		
	Sample Type Acq Method (Dilution	ans1:Comment		
		st:Completed		
		- Inst:DAMethodFileName		
		- Ins1:DAMethodPathName		
		Isi:DataPathName		
		Ins1:Dilution		
		- Ins1:EquilibrationTime		
	Compound ISTD Exp Conc	- 🗐 ns1:InjectionsPerPosition		
		satisfication		
	Second, drag an item from that	- Instanse unendanie		
	same section in the XMI Source	- 🗐 ns1:ISTDDilution 💌		
	man to the Event to bla	< >		
	map to the excertable.	To map repeating elements, drag the elements from the tree onto the worksheet where you want the data headings to appear.		
		To import data, use the Import XML Data button on the List toolbar.		
		Options XML Maps		
		Verify Map for Export		
	/ Design_C	Tips for mapping XML		

Steps	Detailed Instructions	Comments			
	d Click the new column heading, Dilution.				
	e Click the Font Color button on the toolbar.				
	f Select Automatic from the Color dropdown menu.				
 3 Save the new template. Use the filename Quantreport_ISTD_custom_01. xlt. Hint: The filename must be double-quoted. 	 a Select File > Save As. The system displays Save As dialog box. b Type the file name Quantreport_ISTD_custom_01.xlt in the File name text box. Make sure the filename is double quoted in the dialog box. c Click Save to close the Save As dialo box and save the modified template. 	n og			
	Save As	A - M R X R + Tools + A			



Steps	Detailed Instructions	Comments			
4 Generate a new ISTD report in the folder, Test_01-1.	 a To exit Excel, select File > Exit. b Select Report > Generate. The system opens the Report dialog box. c Change the Report Folder from \ Test_01 to Test_01-1. d Click New. e Select Quantreport_ISTD_Custom_01.xlt, and click Open. This step assumes that the prog is still running. If not, see Task is step 1. 				
	Report Graphics Files: © Generate Graphics Files Batch Folder: C\QuanData\DrugsOfAbuse\ Batch File: Batch File: II:Test_01batch.xml O Use Existing Graphics Files Graphics Settings: Local Graphics Settings File Instrument Type determining numeric formats in graphics file Report Eolder C:\QuanData\DrugsOfAbuse\QuantReports!jii_Test_01-2 Reports: Template C:\Mass\QuantReport_ISTD_Custom_011XLT New Delete Localize: f Click OK in the Report dialog box to begin generating the report. g Select Report > Queue Viewer to monitor the progress of report generation. The system displays the Task Queue	Printer tReport_ISTD_Custom_01.xls OK Cancel			

Steps		Detailed Instructions		Comments		
5 Make sure that the Dilution column appears in the new ISTD report.		a Go to the directory\Quantdata\ DrugsOfAbuse\QuantReports\ Test_01-1. b Double-click Quantreport ISTD custom 01.xls.				

🛛 Microsoft Excel - QuantReport_	ISTD_Custom_01.xls							
Eile Edit View Insert F	ormat <u>T</u> ools <u>D</u> ata <u>W</u> indow <u>R</u> eport Desi	gn <u>H</u> elp Ado <u>b</u> e PDF					Type a question for help 🛛 🚽 🖉 🗙	
0 🗃 🖬 🖪 🖨 🖪	🏹 🕺 🖻 🖻 – 🏈 👘 🚪	Tahoma 🔻 10	- B	U		* * * *	& ぷ! 津 津 田 • 🖄 • 🗛 • 📘	
Process Report	ults Add Data 🗣 Add Graphics 📲 Add	Formatting 🔆 Advanced Prone	rties 📕 V	alidate D	esian 🕜 🔞			
- The second provide a se								
A8 🗸	f Sequence Table							
ABCDEF	GHIJKLMNO	PQRSTU	VW	XY	Z AA AB	AC AD AE AF	AG AH AI AJ AK AL AM AN	
8 Sequence Table								
		11.0001010					the second second second second second second second second second second second second second second second s	
10 Data File	Sample Name	Position	Volume	Level	Sample Type	Acq Method File	Dilution	
11 CMAMBIk_01.d	Blank-1	P1-C1	5.0000		Blank	APCIautotune.m	1	
12 CMAMCal_L1.d	Calib-L1	P1-C6	5.0000	L1	Calibration	APCIautotune.m	1	
13 CMAMCal_L2.d	Calib-L2	P1-C10	5.0000	L2	Calibration	APCIautotune.m	1	
14 CMAMCal_L3.d	Calib-L3	P1-C11	5.0000	L3	Calibration	APCIautotune.m	1	
15 CMAMCal_L4.d	Calib-L4	P1-C14	5.0000	L4	Calibration	APCIautotune.m	1	
16 CMAMCal_L5.d	Calib-L5	P1-C17	5.0000	L5	Calibration	APCIautotune.m	1	
17 CMAMQC_L2.d	QC-L2	P1-C9	5.0000	L2	QC	APCIautotune.m	1	
18 CMAMQC_L4.d	QC-L4	P1-C15	5.0000	L4	QC	APCIautotune.m	1	
19 CMAMSam_01.d	Sample-1	P1-C22	5.0000		Sample	APCIautotune.m	1	
20 CMAMSam_02.d	Sample-2	P1-C8	5.0000		Sample	APCIautotune.m	1	
21 CMAMSam_03.d	Sample-3	P1-C12	5.0000		Sample	APCIautotune.m	1 -	
22								

- c Verify that the new column you added, Dilution, appears in the Excel spreadsheet.
- d Select File > Exit.
www.agilent.com

In This Book

The Familiarization Guide presents exercises to help you use the Agilent 6410 Triple Quad LC/MS system. In this guide you learn:

- How to develop an acquisition method
- How to set up and quantitate a batch of Agilent Triple Quad LC/MS data files
- How to inspect your quantitation results, and how to spot irregularities
- How to improve result accuracy
- How to generate and review quantitation reports

© Agilent Technologies, Inc. 2006-2007

Printed in USA Third Edition, August 2007



G3335-90021

