

Agilent MassHunter Workstation – Data Acquisition for 6400 Series Triple Quadrupole LC/MS

Familiarization Guide

Before you begin 3 Prepare your system 3 Prepare to acquire data 4 Exercise 1 – Develop an acquisition method 6 Task 1. Enter acquisition parameters and acquire data 6 Task 2. Determine precursor ion masses 11 Task 3. Find optimum fragmentor voltage for maximum response 14 Task 4. Determine product ion masses 24 Task 5. Find optimum collision energy for MRM acquisition 30 Exercise 2 – Develop a Dynamic MRM acquisition method from an MRM acquisition data file or an MRM method 33 Task 1. Create a batch file from an existing MRM data file 33 Task 2. Print a report in the Quantitative Analysis program 36 Task 3. Create a Dynamic MRM method using Update dMRM 37 Task 4. Create a Dynamic MRM method from an MRM method 39 Exercise 3 – Create a Triggered Dynamic MRM acquisition method 40 Task 1. Create a Triggered Dynamic MRM method from a Dynamic MRM method manually 40 Task 2. Add/Modify compounds in an existing database 42 Task 3. Create a Triggered Dynamic MRM method from an existing database 52 Exercise 4 – Optimize Acquisition parameters 56 Task 1. Use the Optimizer Software to optimize acquisition parameters 56 Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters 63



Use the exercises in this guide to learn how to use the Agilent 6400 Series Triple Quad LC/MS. You can do these exercises with the demo data files, SulfaDrugs, shipped with the system (in the **Data** folder of your Qualitative Analysis installation disk), or with data you acquire.

In Exercise 1, you learn how to determine the best 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 Qualitative Analysis program to identify parameter values producing optimum signal response. You can also learn about the Qualitative Analysis program by using the *Qualitative Analysis Familiarization Guide* or the Qualitative Analysis online Help.

In Exercise 2, you learn how to use either an acquired data file or the Quantitative Analysis report results to update a dynamic MRM method. This method allows you to easily set up a dynamic MRM method.

In Exercise 3, you learn how to create a triggered dynamic MRM method.

In Exercise 4, you learn how to use two programs to optimize parameters. The Optimizer Software helps you optimize acquisition parameters. Specifically, it automates the selection of the best precursor ion and the fragmentor voltage for the most abundant precursor ion, selection of the best product ions, and optimization of collision energy values for each transition for a list of compounds you specify. The "Source and iFunnel Optimizer" program helps you to find the optimal source and iFunnel parameters.

NOTE See the *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 task 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

Before you begin, you need to check that your system is ready. If you plan to acquire data, you also need to set up the instrument.

Prepare your system

- 1 Check that:
 - The Data Acquisition program has been installed.
 - The LC modules and the 6400 Series Triple Quad LC/MS have been configured.
 - The performance has been verified.
 - The system has been turned on.

If these actions have not yet been done, see the *Installation Guide* for your instrument.

2 Copy the data files to your PC.

Copy the folder named **SulfaDrugs** in the **Data** folder on your Qualitative Analysis installation disk 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 disk and you are the only one using them. Data files that are already on the system may contain processed results, leading to different behavior during the exercises in this guide.

Prepare to acquire data

If you do not intend to acquire data but want to learn how to use the Qualitative Analysis program for method development, you can skip this step, which tells you how to prepare the demo sample. You then do those tasks that show you how to use the Qualitative Analysis program with the sulfa drug data files shipped with the system.

Parts List The exercise in this guide uses this equipment and materials:

- Agilent 1200, 1260 Infinity or 1290 Infinity LC modules: well-plate sampler, binary pump, thermostatted column compartment, DAD
- Zorbax column (see Table 1 on page 4)
- A 1 ng/µL concentration of the sulfa mix sample (prepared in this step)

Table 1Zorbax columns

| Triple Quadrupole | Column Description | Film Thickness | Pore Size Part Number | |
|-------------------------------------|---------------------|-------------------|--------------------------|------------|
| 6410B, 6420, 6430, 6460 and 6490 | SB-C18 2.1mm x 50mm | 1.8 µm | 80Å | 822700-902 |

1 Prepare the LC solvent.

In 1-liter reservoirs of HPLC-grade water and acetonitrile (ACN), add 1 mL of 5M ammonium formate each to make 5mM ammonium formate in water and ACN and use for the A and B channels, respectively.

- **2** Prepare the sample.
 - **a** Add 10 μ L of the sulfa mix from one of the ampoules (500 μ L) to 990 μ L of solvent A in a 2 mL glass sample vial so that the final concentration is 1 ng/ μ L.
 - **b** Cap the vial and place in a sample location in the autosampler.
- **3** Set up the LC column.

Use the Agilent column from Table 1. Other columns and instrument parameters may be used in these exercises, but some parameters may need adjustment, and the results will differ.

4 Set the column temperature to 60°C. Lower temperatures may be used; however, the retention times will be longer, and the pump pressure may exceed the limit of some LC systems.

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 *Quick Start Guide, Installation Guide* or online Help for instructions on tuning the instrument.

Task 1. Enter acquisition parameters and acquire data

Exercise 1 – Develop an acquisition method

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

Task 1. Enter acquisition parameters and acquire data

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

| Steps | | | Detailed Instructions | | Comments | |
|-------|---|--|--|---|--|--|
| 1 | Enter LC parameters appropriate for sulfa drug mix. | a Double-click the Data Acquisition icon. b Make sure that Acquisition appears as | | • | The Data Acquisition window appears. See Figure 1. | |
| | See Table 2. | C | the selection in the Context text box. If Tune is the selection, click Acquisition from the Context dropdown menu in the Combo bar. Enter the LC parameters listed in the Table 2. | | | |

Table 2 LC parameters for sulfa drug mix

| Parameter | LC Parameter |
|-----------------------|---|
| PUMP | |
| Flowrate | 800 μL/min |
| Solvent A | 5 mM ammonium formate in water |
| Solvent B | 5 mM ammonium formate in 90:10 acetonitrile:water |
| • Gradient (min - %B) | 0 min - 13% 1.80 min - 60% 2 min - 60% |
| • Stop Time | 2.5 min |
| Post Time | 3.0 min |
| INJECTOR | |
| • Inj. Vol. | 2.0 μL |

Task 1. Enter acquisition parameters and acquire data

| Parameter | LC Parameter |
|--------------------|--|
| Injection | Standard |
| Draw Position | 0.0 mm |
| UV DETECTOR | |
| • Ch A | 254 nm (4 nm BW on DAD) |
| • REF A (DAD only) | 400 nm (80 nm BW) |
| COL THERM | |
| • Temp | 60 °C for the 6460 and 6490 with Agilent Jet Stream Technology |
| | 40 °C for other instruments |

Table 2 LC parameters for sulfa drug mix (continued)

Task 1. Enter acquisition parameters and acquire data

| Agilent MassHunter Workstation D | Data Acquisition | | | | | | |
|--|--|--------------------------|-------------------|--------------------|-----------------------|-------------------------------------|------------------|
| File View Sample Worklist | Method Tools Help | | | | | | |
| Context: Acquisition • La | yout newleyout.lyt 🔹 💽 - 🧯 | | I Method | pfhMS2Scantest.m | | | • Worklist: |
| Instrument Status | | | | | | × Actuals | × |
| 📀 🛛 HiP Sampler 👝 🗖 | 🍵 Binary Pump 🔔 🔳 🏏 | Column Comp. | _ = 🔽 D/ | | QQQ | Parameter | Value |
|) Ø Idle | i) el lo |) I die | U) ENIO | Idle |) Idle | QQQ: Not Ready Text Long | |
| 4 | R R | | 0 | in i | | QQQ: Collision Gas | on 25.0 mil |
| | | | 3 | Line | AIS ESI | Gale. House | acopa |
| 2.0µL | 87.0 13.0 | | | 6 | C 19 | | |
| 2 | 0.800 ml/min | | * | | | | |
| | 1001/1 Dat | | | | | a | |
| | | 59.96 °C 23. | 55 °C | | UV 8 | | |
| | | | | | | | |
| 192.168.254.11 0.0 | 0 / 0.00 | | | Instrument Idle | 🥐 Θ On | <u>0</u> он | |
| Chromatogram Plot | | | , | Spectrum Plot | | | × |
| | TIC | | | 000 Spectrum | MS 1: MS2 Scan, ESI+/ | Aqilent Jet Stream (+), 58.97 | |
| Unin | | | | 6 4000 Area: 0 | 9 | | |
| 7500000 | | | | 2000 FWHM. | 0.7 | | 348.9 |
| 2500000 | | | | A Height: 3 | 178 | | 380.8 396 |
| | ~ ~ ~ ~ | | | 100 | 150 | 200 250 200 | 350 400 |
| , <u>111</u> | 20 30 | | ov min | A A | 19970 | m/z(amu) | |
| Method Editor | | | | | | | × |
| E pfhMS2Sc | antest.m | | Apply 🔛 | | | | |
| Properties DA HiP Sampler | HIP Sampler Pretreatment Binary Pump | Column Comp. D | AD QQQ | | | | |
| | | | | | | Column | n Comp. (G1316A) |
| Temperature | | + Advanced | | | | | |
| Left | Right | Foundation of the second | | | | | |
| Not Controlled | Not Controlled | Chable Modylin | Left | | | Bete | |
| e 60.0 ; °C | 0 20.0 C TC | | A life and have a | | | Nith an instant | |
| | Combined | | with any temper | arure | | with any temperar | ue - |
| | | | when temperatu | re is within | | When temperature | 1 WORD |
| Sector Se | 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1 | | 1.0 | 101 · 1 · 10 | | 1 00 | |
| Stoplene | Postane | | | | | | |
| As PumpAniector | a 04 | | | | | | |
| 100.1 min | 100.5 min | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | • Timetable | | | | | |
| WorkList Method Editor Sample F | lun | | | | | | |

Figure 1 Agilent MassHunter Workstation Software – Data Acquisition window

| Steps | | De | etailed Instructions | Comments | |
|-------|--|----|---|----------|--|
| 2 | Enter MS parameters appropriate for sulfa drug mix and save the | a | Click the QQQ tab in the Method Editor window. | | |
| | method as <i>iii</i> MS2Scantest.m, where <i>iii</i> are your initials. | b | Select MS2Scan from the Scan Type list in the Time Segments table. | | |
| | | C | Enter the other MS parameters as | | |
| | See Table 3. | | listed in Table 3. These parameters are in either the Acquisition or the Source tabs. | | |
| | | d | Save the method as <i>iii</i> MS2Scantest.m, where <i>iii</i> are your initials. | | |

Task 1. Enter acquisition parameters and acquire data

| Parameter | Value (ESI) | Value (AJS ESI) |
|----------------------------|---|---|
| • Inlet | ESI (positive polarity) | AJS ESI (positive polarity) |
| • Scan Type | MS2Scan | MS2Scan |
| • Delta EMV pos | 400 V | 200 V |
| • Mass Range | 100 to 400 | 100 to 400 |
| Cell Acceleration Voltage | 7 V | 7 V |
| • Gas Temp | 350 °C | 350 °C |
| | 250 °C for Agilent 6490 | 250 °C for Agilent 6490 |
| • Gas Flow | 12 L/min | 10 L/min |
| | 14 L/min for Agilent 6490 | 14 L/min for Agilent 6490 |
| • Nebulizer | 50 psi | 35 psi |
| Sheath Gas Temperature | not applicable | 400 °C |
| Sheath Gas Flow | not applicable | 12 L/min |
| Nozzle Voltage | not applicable | 0 V |
| Capillary Voltage positive | 4000 V | 4000 V |
| • Fragmentor | 100 V (not adjustable on 6490, comes from the Tune file) | 100 V (not adjustable on 6490, comes from the Tune file) |

Table 3MS parameters for sulfa drug mix



Figure 2 Select Scan Type of MS2 Scan in the QQQ 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 View > Worklist to display the Worklist window. b Click Worklist > Worklist Run Parameters. Verify that the parameters are set properly. Click OK. c Click Worklist > Add Multiple Samples. d Type <i>iii</i>sulfamix01.d as the data file name e Select <i>iii</i>MS2Scantest.m as the method name. f Click the Sample Position tab. g Select the Autosampler, Well-plate or Vial Tray. h In the graphic, select a single position. Click OK. i In the Worklist window, mark the check box to the left of the sample. | The Worklist window is tabbed with the Method Editor window by default. Click the Worklist tab to show the Worklist window. The Number of samples is set to 1. 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 program. If you prefer, you can create your own data file as described in this step. | | |
| | | | | |

| | ĉ I | 📕 🛃 🕨 💷 💷 💆 | • | | |
|---|--------------------|-----------------|-----------------|------------------|-----------------|
| | 7 | Sample Name | Sample Position | Method | Data File |
| 1 | $\boldsymbol{\nu}$ | Sample 1 | Vial 1 | pfhMS2Scantest.m | pfhsulfamix01.d |

j Click the Start Worklist Run icon in the main toolbar, the Run Worklist icon in the Worklist toolbar or click the Worklist > Run command.

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 | Comments | |
|--|--|---|--|
| Open the acquired data file. In the 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 6. | a Double-click the Qualitative Analysis icon. The program 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. Clear this check box before opening the file. | |



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 6, and click Open. By default, the system displays the Total Ion Chromatogram (TIC). | The figure below shows the default layout. 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. | | |

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

- Restore default layouts
 - Click Configuration > Window Layouts > Restore Default Layout.
- Make sure the method is default.m. (see title bar)
 - Click Method > Open.
 - Select default.m, and click Open.
- Return display options to default settings.
 - In the Configuration menu, click each of the Display Options commands.

• Click Default, and then OK. Or...

- Restore the General layout.
 - Click Configuration > Configure for Workflow > General.
 - Click OK.
 - (optional) You may be asked to save method changes.
- Return display options to default settings.
 - In the Configuration menu, click each of the Display Options commands.



Task 2. Determine precursor ion masses

| Steps | | | | Detailed Instructions | | Comments | | |
|-------|---|-------------------------------|--|-----------------------|---|--|-----------------------------|--|
| 2 | Determine precursor ion masses for all four peaks. • You have determined them correctly if you find the values are similar to those shown in this table: | | | a b | 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 figure below. | The system displays an averaged spectrum across the peak in the M Spectrum Results window. The precursor mass of the first compound, sulfamethizole, is determined to be m/z 270.9. To obtain a single scan, double- | | |
| | Compound | RT m/z | | C C | Right-click the shaded area, and click | | click the apex of the peak. | |
| | Sulfamethizole Sulfachloropyridazine | 0.47 270.9 zine 0.88 284.9 | | shortcut menu. | | | | |



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 Click 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)+.

Agilent 6400 Series Triple Quad LC/MS Familiarization Guide

•

•

Sulfamethazine

Sulfadimethoxine

1.20

2.23

If you acquired the data file using

Technology, the retention times

• The sulfamix01.d data file was

so your retention times are

the precursor ion masses.

Close the data file after finding

the Agilent Jet Stream

may be different.

different.

279.0

311.0

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.

The Fragmentor Voltage for the 6490 is set automatically during Autotune, and it cannot be set in the Data Acquisition program. If your instrument is a 6490, skip to "Task 4. Determine product ion masses". You can do the Qualitative Analysis part of this task by using the data files that were shipped with the software.

| Steps | Detailed Instructions | Comments |
|--|---|--|
| Set up six methods for six different fragmentor voltages. Change to a SIM experiment. Use 60, 80, 100, 140, 180 and 220 | a In the Scan Type dropdov MS2 SIM. | /n list, click |
| Use 60, 80, 100, 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. | Tune file atunes.tune.xml Browse Gon source ESI Agilent Jet Stream | Stop time No limit/As Pump Time filtering Peak width 0.07 min |
| | Time segments # Start Scan Type # Start Scan Type 1 0 MS2 SIM MS2 SIM MS2 Sim MS2 Sim MS2 Sim MS2 Sim MRM Product Ion Precursor Ion Neutral Loss 4 91 cyclesry TyTA 5 | Div Valve Delta EMV (+) Stored To MS 0 0 i |

| Detailed Inst | ructions | 5 | | C | Comments | | | | | |
|---|--|----------------------|---------------------------------|----------------------------|---------------------------------|--|--|--|--|--|
| b In the Acq Compound (precursor sulfadimet c Right-click segments d Type the C Mass for s e Repeat ste sulfametha f Save the n where <i>iii</i> a g Change the and save the <i>iii</i>MS2SIW initials. h Repeat ste and 220, sa <i>iiii</i>MS2SIW <i>iiii</i>MS2SIW where <i>iii</i> a | b In the Acquisition tab, enter the Compound Name and Mass (precursor ion mass) for sulfadimethoxine. c Right-click anywhere in the Scan segments section, and click Add Row. d Type the Compound Name and the Mass for sulfachloropyridazine. e Repeat steps c and d for sulfamethazine and sulfamethizole. f Save the method as <i>iii</i>MS2SIM140.m, where <i>iii</i> are your initials. g Change the fragmentor voltage to 60, and save the method as <i>iii</i>MS2SIM060, where <i>iii</i> are your initials. h Repeat step g for voltages 80, 100, 180 and 220, saving the methods as <i>iii</i>MS2SIM180 and <i>iii</i>MS2SIM120, <i>iii</i>MS2SIM180 and <i>iii</i>MS2SIM220, where <i>iii</i> are your initials. | | | | | With the MS2SIM Scan Type set, a different set of columns appears in the Acquisition window. The Instrument Control and Data Acquisition program creates a SIM experiment for each compound mass, starting with a default fragmentor voltage of 140. See the example below. The Fragmentor column is grayed out if the instrument type is an Agilent 6490. | | | | |
| Acquisition Source | Chromatogram | n Instrument | Diagnostics | | | | | | | |
| Community I | | | | | | | | | | |
| Scan segments Compound Name | ISTD? | Mass 🗸 | MS2 Res | Dwell | Fragmentor | Cell Accelerator Voltage Polarity | | | | |
| Scan segments Compound Name sulfadimethoxine | ISTD? | Mass V 311 | MS2 Res Unit | Dwell 200 | Fragmentor 140 | Cell Accelerator Voltage Polarity 7 Positive | | | | |
| Scan segments Compound Name sulfadmethoxine sulfachloropyridazine | ISTD? | Mass 7 311 285 | MS2 Res Unit Unit | Dwell 200 200 | Fragmentor 140 140 | Cell Accelerator Voltage Polarity 7 Positive 7 Positive | | | | |
| Scan segments Compound Name sulfadimethoxine sulfachloropyridazine sulfamethazine | ISTD? | Mass 311 285 279 | MS2 Res Jnit Jnit Jnit | Dwell 200 200 200 | Fragmentor 140 140 140 | Cell Accelerator Voltage Polarity 7 Positive 7 Positive 7 Positive | | | | |

| Steps | Detailed Instructions | Comments |
|---|--|--|
| 2 Set up and run the worklist (optional). Set up six samples with Sample Name SulfaDrugMix to inject 1ul 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 xxx is the voltage. | a Click the Worklist icon if necessary to make sure the worklist is visible. b Click Worklist > New to start a new worklist. You do not need to save the last worklist. c To set up the run, right-click the upper left corner of the worklist, and click Worklist Run Parameters. d Type the paths for the method and data files. e Type the information for the 60 voltage run. f Click Worklist > Add Sample. Another sample is added to the Worklist. Add five samples to the worklist for voltages 80-220. g Mark the checkbox to the left of the Sample Name for each of the six samples. | This step is optional because you can use data files shipped with the system to perform many of the tasks in this exercise. |
| | WorkLit Sample Name Sample Position Methin Sample 1 Vial 1 pthMS2SIM60 pthMS2M50 pthMS2M50 pthMS2M60 pth | Jd Date File Sample SuiteSim60.d Sample SuiteSim60.d Sample n SuiteSim100.d suiteSim100.d Sample n SuiteSim100.d Sample Sample n SuiteSim100.d SuiteSim200.d Sample n SuiteSim100.d Sample Sample n SuiteSim200.d Sample Sample Note that the program only runs those samples that are marked with a checkmark. • You can also run the worklist in locked mode by clicking the Image: Comparison of the sample suite of the sa |

Task 3. Find optimum fragmentor voltage for maximum response

| Steps | Detailed Instructions | Comments | | |
|--|---|--|--|--|
| 3 Set up a qualitative method to view the EIC data automatically. Open the data file Sulfa_SIM60.d or your own <i>iii</i>Sulfa_SIM60.d, where <i>iii</i> are your initials. In the Method Editor, add in the EICs corresponding to the precursor ion masses of 271, 279, 285, and 311. | a Click File > Open Data File. The system displays the Open Data File dialog box b Select either Sulfa_SIM60.d or <i>iii</i>Sulfa_SIM60.d, and click Open. c Click Method > Method Editor or View > Method Editor. The system displays the Method Editor window. | The Qualitative Analysis program should be open. If not, see "Double-click the Qualitative Analysis icon." on page 11. | | |
| Save the method as <i>iii</i>Evercise1 | | | | |

 Save the method as *iii*Exercise1, where "*iii*" are your initials.



| Steps | Detailed Instructions | Comments |
|-------|--|--|
| | d If necessary, click Define Chromatograms in the Chromatogram section of the Method Explorer. e To delete the BPC chromatogram, click Delete. f Select EIC for the Chromatogram Definition Type, g In the MS Chromatogram tab, make sure MS Level is set to All and Scans is set to All scan types. h Clear the Do cycle sum check box. i Type 271 as the m/z value. j Click Add. k Repeat steps i and j for the other precursor ions, 279, 285 and 311. I Click Method > Save As. The system opens the Save As dialog box m Save the method as <i>iii</i>Exercise 1.m. n Click Save. | The default Method Editor list selection after installation is Integrate (MS). You can also select Define Chromatograms from the Method Items list in the Method Editor window. |
| | Method Editor: Define Chromatograms Extract Defined Chromatograms Defined chromatograms Eic (27) 00000 m/2) Al Eic (280 0000 m/2) Al Eic (280 m/2) Al Mice and when extracted and the provided and the | |

| Steps | Detailed Instructions | Comments | | |
|--|---|--|--|--|
| 4 Extract the chromatogram for the data file and view the results. Make sure you can see all five | a Click the Run button on the Method Editor toolbar. | You can also click the Chromatograms > Extract Defined Chromatograms command to | | |
| chromatograms, the TIC and four | Method Editor: Define Chromatograms | extract the defined chromatograms. | | |
| Elüs. | 💽 Extract Defined Chromatogram 🔹 🚮 🖃 🕫 🖓 | -1 | | |
| | b To see the TIC and four EICs, click the arrow next to the Maximum Number of List Panes icon in the Chromatogram Results toolbar, as shown in the example below. c Select 5 to view five chromatograms simultaneously. The system displays chromatogram results as shown below. | | | |
| | | | | |
| | :// Chromatogram Results : य ↔ ‡ ♀ (⊉ २४/४४) ▲ ೨ ० ा ♥ ₩ 🔝 🛦 /໓ | 🗶 🧏 % % 🎉 📼 Minutes 💌 🍙 | | |
| | x10 ⁶ + TIC SIM Sulfa_SIM60.d 3 4 | | | |
| | x10 ⁵ + EIC(271.00000) SIM Sulfe_SIM60.d 7 8 | <u>, , , , , , , , , , , , , , , , , , , </u> | | |
| | x10 ⁶ + EIC(279.00000) SIM Sulfa_SIM60.d 9 10 + | | | |
| | 0 x10 ⁵ + EIC(285.00000) SIM Sulfa_SIM60.d | | | |
| | x10 ⁶ +EIC(311.00000) SIM Sulfa_SIM60.d | | | |
| | Counts vs. Acqu | uisition Time (min) | | |

| 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, Sulfa_SIM80.d through Sulfa_SIM220.d. | a Select File Open Actions from the General section in the Method Explorer. b Make sure that Actions to be run list only contains Extract Defined Chromatograms. | The Qualitative Analysis Method Editor lets you define actions to be performed automatically upon opening a data file(s). |
| Close the Method Explorer. | Method Editor: Assign Actions to Run Opening a Da Available actions Extract Defined Chromatograms Compound Automation Analysis Automation Find Compounds by Aarot MS/MS Find Compounds by Targeted MS/MS Find Compounds by Fargeted MS/MS Find Compounds by Fargeted MS/MS Generate Compound Report Generate Analysis Report Generate Formulas from Compound Extract Defined Chromatograms | |
| | c Click File > Open Data File. The system displays the Open Data File dialog box. d Select the data files to be opened, Sulfa_SIM80.d through Sulfa_SIM220.d. e Mark the Run 'File Open' actions from selected method check box. (lower left corner) | |

| Steps | Detailed Instructions Comments |
|-------|--|
| | Den Data File |
| | Look in: 🔒 SulfaDrugs 🔽 🔘 🎓 🖽 |
| | QuartReports Sufe QuartReports Sufe QuartReports Sufe Sufe_SIM100.d Sufe_SIM100.d Sufe_SIM100.d Sufe_SIM20.d Sufe_SIM20.d Sufe_SIM20.d Sufe_SIM20.d Sufe_SIM20.d Sufe_SIM20.d Sufe_SIM20.d Sufe_SIM20.d Sufe_SIM20.d Sufe_MontMIM_10.d SufemoNRM_20.d SufemoNRM_20.d Sufe_SIM10.d.""Sufe_SIM10.d.""Sufe_SIM10.d.""Sufe_SIM10.d.""Sufe_SIM10.d." Open File name: Sufe_Simple Name: User current method User Name : SufePosition |
| | f Click Open. 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. You can also close the Method Explorer and Method Editor windows by clicking the View > Method Explorer command and the View > Method Editor command. |

Task 3. Find optimum fragmentor voltage for maximum response

Steps

Detailed Instructions

Comments



Task 3. Find optimum fragmentor voltage for maximum response

| 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/ b Click the Show only the highlight items icon, . Donly the 271 m/z check boxes marked. c Look at the relative intensities peak to determine which fragm voltage setting will be best to the 271 precursor. | You press the Ctrl key to be able to select multiple objects from the Data Navigator window. You press the Shift key to be able to select a group of objects. 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 |
| | Sot by Data File | Q █ ₩ V <mark> </mark> <mark> </mark> |
| | ▼ User Chromotyrans x10 ³ = BC7 ▼ Wiser Chromotyrans 64 ● A = BC275 00000 SM 64 ● A = BC275 00000 SM 65 ● Beckground Spectra 58 ● Orse Sectra 58 ● Ø User Chromotyrans 54 ● Ø User Chromotyrans 55 ● Ø User Chromotyrans 55 ● Ø User Chromotyrans 54 ● Ø User Chromotyrans 54 <t< td=""><td>You can overlay the chromatograms by clicking the Overlaid mode icon in the Chromatogram Results toolbar.</td></t<> | You can overlay the chromatograms by clicking the Overlaid mode icon in the Chromatogram Results toolbar. |
| | d Repeat step a through step c f | or the |
| | other three base peaks or prec | eursor |
| | ions. e Click File > Close Data File. f Click Close when the Close Da dialog box appears. | Click the different EICs in the Data Navigator window to change which chromatogram is labeled in the Chromatogram Results window. When the color of the label of the chromatogram matches the color of |

the chromatogram that has the highest intensity, you use the fragmentor voltage that was used

for that file.

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) acquisition.

| Steps | Detailed Instructions | Comments | | |
|---|---|---|--|--|
| 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 Pl_xx.m, where <i>iii</i> are your initials and xx is the collision energy. | a Click the QQQ tab in the Method Editor pane. b Select Product Ion in the Scan Type combo box to scan each precursor ion for all its product ions. c Enter all MS parameters as listed in the example below, making sure the Collision Energy is set to 15 and the Fragmentor voltage determined in Task 3. d Save the method as <i>iii</i>Sulfamix PI_15.m. e Repeat step c and step d for collision energies of 30 and 45. | When you change the Scan Type in the Time Segments table, the Scan segments table is reset. If you want to copy the Scan segments to the new Scan segments table, highlight all of the lines in the Scan segments table and then right-click the Scan segments table and click Copy. After you select a new Scan Type, right-click the Scan segments table and click Paste from Clipboard. You cannot copy and paste the Scan segments table between all Scan Types. | | |

| atunes.TUNE.XML | No limit/As Pump | -Sca | n segments | | | | | | | | |
|---|--|-------------|-----------------------|-----------------|----------|--------|-----------|------------|------------------|--------------------------|----------|
| Branna LL | C 1 min | | Segment Name | Precursor Ion V | MS2 From | MS2 To | Scan Time | Fragmentor | Collision Energy | Cell Accelerator Voltage | Polarity |
| biowse 00 | | | sulfadimethoine | 311 | 50 | 320 | 250 | 140 | 15 | 7 | Positive |
| Ion source | Time filtering | | sulfachloropytidazine | 285 | 50 | 320 | 250 | 140 | 15 | 7 | Positive |
| AJS ESI 💌 | Peak width 0.07 min | | sulfamethazine | 279 | 50 | 320 | 250 | 140 | 15 | 7 | Positive |
| _ | to contract local | 11 | sullamethizole | 271 | 50 | 320 | 250 | 140 | 15 | 7 | Positive |
| tt Staf ∕ Scan Type Div ≯∫ 1 00 Production ▼ To8 | Valve Defa Defa Defa Socied EMY-19 EMY-19 Socied S 0 0 0 0 0 | -50 | in Daraméters | | | | | | | | |
| | | Step | o size: 0.1 | · · · . | umu | | | | | | |
| 1 cycles/s 913.1 ms/c | rcle | Dat. The | eshold: | ofile 💌 | | | | | | | |

- 2 Set up and run the worklist (optional).
 - Specify the data files as *iii*Sulfamix Pl_xx.d, where *iii* are your initials and xx is the collision energy.
- a Click the Worklist tab.
- **b** Add three samples to the worklist for collision energies 15, 30 and 45.
- **c** Mark the check box to the left of the Sample Name for each sample you are adding.
- d Click Worklist > Run.

- 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.

Task 4. Determine product ion masses

📝 Do cycle sum

Task 4. Determine product ion masses

| Steps | Detailed Instructions | Comments | | |
|-------|---|---|--|--|
| | n From the Method Explorer in the Chromatogram section, click Integrate (MS/MS). o Select MS/MS as the Integrator selection, if necessary. | These data files contain MS/MS data, so you need to modify the parameters in the Integrate (MS/MS) section. If the data file contained only MS data, you would need to modify the parameters in the Integrate (MS) section. | | |

| Method Editor: Integrate (MS/MS) 🗙 🗙 | | | | | | | | |
|--------------------------------------|--|--|--|--|--|--|--|--|
| 🜔 Integ | rate Chromatogram 🔹 🔛 🖛 🗢 👘 Method Items 🔹 😕 🙀 | | | | | | | |
| Integrator | Suitability Peak Filters Results | | | | | | | |
| Integrator | selection | | | | | | | |
| MS/MS | • | | | | | | | |
| This is | a parameter less integrator. | | | | | | | |

Figure 3 Integrate (MS/MS) > Integrator Tab

p Click the Peak Filters tab. Make sure that the Limit (by height) to the largest check box is marked and set to the value 100 as shown below.

| ntegrator | Suitability | 🛕 Peak Filters | Results | |
|-------------|---------------|----------------|---------|-------------------|
| Filter on | | | | |
| | Peak | height | Peak | area |
| Height filt | ers | | | |
| Absolu | ute height | >= | 10000 | counts |
| Relativ | ve height | >= | 5.000 | % of largest peak |
| Area filten | 5 | | | |
| 📄 Absolu | ute area | >= | 10000 | counts |
| 🗸 Relativ | /e area | >= | 1.000 | % of largest peak |
| Maximum | number of p | eaks | | |
| 🔽 Limit (| by height) to | the largest | Δ | 100 |

Figure 4 Integrate (MS/MS) > Peak Filters tab

Task 4. Determine product ion masses

| Steps | Detailed Instructions | Comments |
|-------|--|---|
| | q Click General in Method Explor then click File Open Actions. r Select Integrate and extract per spectra from the Available actionand click reprint to add this to A to be run. | er, and eak ons list Actions |
| | Figure 5 General > File Open Actions to Run Opening a Data File Image: Specific Speci | thod Items () () () () () () () () () () () () () |
| | s To apply the changes to the cur | rent |

| | | J | method, <i>iii</i> exercise1.m, click the Save Method icon. You can also click Method > Save. | | |
|---|--|---|---|---|---|
| 4 | Run the qualitative method on the current data file. | • | In the Method Editor toolbar, click the Run button, () . When the Assign Actions to Run Opening A Data File section is displayed, the Actions to be run list is executed. | • | The program 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 each integrated peak. See Figure 6 on page 28. |

Task 4. Determine product ion masses



Figure 6 Results for integration and extraction of peak spectra.

- **5** Run the 'File Open' actions 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 Click 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
 DL 20 d and Sulfamix DL 45 d
 - PI_30.d, and Sulfamix PI_45.d.
 Select the data files you acquired in step 2.
- c Mark the **Run 'File Open' actions from** selected method check box 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 each integrated peak.

Task 4. Determine product ion masses

| 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, . c Click View > MS Spectrum Peak List 1. d Examine the spectra to see which fragment ions are produced at which collision energies. e Repeat steps a to d until all the product ions are identified | 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. The peak may not be labeled if the peak is too wide. | | |



f Click the Close Data File icon in the main toolbar, 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 > 155.729 Sulfadimethoxine-311.0 > 155.7

Agilent 6400 Series Triple Quad LC/MS Familiarization Guide

Task 5. Find optimum collision energy for MRM acquisition

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 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, 25, 30 and 35 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. | | |

| Tune file Stop time | Acc | quisition Source | Chromato | gram Instrumer | t Diagno | stics | | | | | | |
|--|-----|-----------------------|----------|-----------------|----------|---------------|---------|-------|------------|------------------|-----------------------------|----------|
| Browse 66 C 1 min | | Compound Name | ISTD? | Precursor Ion 🗸 | MS1 Res | Product Ion V | MS2 Res | Dwell | Fragmentor | Collision Energy | Cell Accelerator Voltage | Polarity |
| lon source | | Sfuladimethoxine | | 311 | Unit | 155.7 | Unit | 50 | 100 | 10 | 7 | Positive |
| ESI T Dark with 0.07 min | | Sulfachloropyradizine | | 285 | Unit | 155.7 | Unit | 50 | 100 | 10 | 7 | Positive |
| | | Sulfamethazine | | 279 | Unit | 185.7 | Unit | 50 | 100 | 10 | 7 | Positive |
| Time segments | 2 | Sulfamethizole | | 271 | Unit | 155.8 | Unit | 50 | 100 | 10 | 7 | Positive |
| tt Scan Type Dir Valve Dir Mark Dir Mark Dir Mark Scan Type 1/2 0 MRM To MS 18 0 IV 467 cyclex/s 214.0 ms/cycle 1467 1214.0 1467 | | <u>د </u> | | | | | | | | | | |

- 2 Set up and run the worklist (optional).
 - Specify the data files as *iii*Sulfamix MRM_xx.d, where *iii* are your initials and xx is the collision energy.
- **a** Click the **Worklist** tab to make the worklist visible.
- **b** Add six samples to the worklist for collision energies 10, 15, 20, 25, 30, 35.
- c Mark the checkbox to the left of the Sample Name for each of the three samples.
- d Click Worklist > Run.

• This step is optional because you can use the six example data files in the next step.

Task 5. Find optimum collision energy for MRM acquisition

| St | eps | Detailed I | nstructions | C | Comments | | |
|----|---|---|--|---|---|--|--|
| 3 | Compare the compound transition intensities at different collision energies. • Open the MRM data files: SulfamixMRM_10.d SulfamixMRM_20.d SulfamixMRM_20.d SulfamixMRM_25.d SulfamixMRM_30.d SulfamixMRM_35.d • 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 | Open ti program Clear ti check li Open ti Qualita Right-co window Chrom menu. To sele while his Enter ti exampli Clear ti MRM co | he Qualitative Analysis m. he Run 'File Open' actions box. he MRM data files in the itive Analysis program. click the Chromatogram Resu w, and click Extract atograms from the shortcut ct all data files, click the last nolding down the Shift key. he parameters as listed in th le below, and click OK . he TIC check boxes to make chromatograms easier to view | • Ilts file e the w. | 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. | | |
| | one collision energy with the same compound transition obtained at another collision energy. (Do this in Overlaid Mode with all the MRM chromatograms.) Close the data files but don't save results. Refer to Table 4 on page 32 for optimal method settings for each compound. | Extract Chrom List of opened of SulfamixMRM SulfamixMRM SulfamixMRM SulfamixMRM SulfamixMRM | atograms tata files 10 d 15 d 20 d 20 d 25 d 30 d 35 d MRM MS Chromatogram MS level: Scans: Transition: A | Advanced AS/MS V Autiple reaction | | | |

- h Click the Overlaid Mode icon, 🔼
- i Compare peak intensities for each compound transition in each data file in the Chromatogram Results window.
- Compare the colors shown in Chromatogram Results with the color next to the MRM transition name in the Data Navigator.

Cancel

ОК

 You can also right-click the Chromatogram Results window header and compare the colors of the chromatograms to the colors of the titles in the shortcut menu.

Task 5. Find optimum collision energy for MRM acquisition



Unless you decide to acquire MRMs at lower collision energies, you should find that the optimal method settings are as shown in Table 4.

- j Click the **Close Data File** icon in the main toolbar, 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.

Table 4 Compounds and Collision Energy

| Compounds | MRM Transition | Collision Energy (V) | |
|-----------------------|----------------|----------------------|--|
| Sulfamethizole | 271.0 > 155.8 | 10 | |
| Sulfamethazine | 279.0 > 185.7 | 15 | |
| Sulfachloropyradizine | 285.0 > 155.7 | 10 | |
| Sulfadimethoxine | 311.0 > 155.7 | 15 | |

Task 1. Create a batch file from an existing MRM data file

Exercise 2 – Develop a Dynamic MRM acquisition method from an MRM acquisition data file or an MRM method

The purpose of this exercise is to create a Dynamic MRM method from an acquired MRM data file for sulfamix_MRM data files with the correct retention times for Dynamic MRM using the Quantitative Analysis program.

For this exercise, you have three main tasks:

- "Task 1. Create a batch file from an existing MRM data file" on page 33
- "Task 2. Print a report in the Quantitative Analysis program" on page 36
- "Task 3. Create a Dynamic MRM method using Update dMRM" on page 37

You can easily create a Dynamic MRM method from an existing MRM method.

• "Task 4. Create a Dynamic MRM method from an MRM method" on page 39

Task 1. Create a batch file from an existing MRM data file

In this exercise, you create a batch and a method from an existing MRM data file.

| Steps | Detailed Instructions | Comments | | |
|---|---|---|--|--|
| Open the Quantitative Analysis program and create a batch file with one sample file, SulfamixMRM_35.d. Copy the data file SulfamixMRM_35.d from the installation disk to the \MassHunter\Data\MRM_to_ DMRM folder. | a Double-click the QQQ Quantitative Analysis icon. b Click File > New Batch. c Navigate to the \MassHunter\Data\ MRM_to_DMRM folder. d Type MRM_to_DMRM in the File Name text box. e Click Open. f Click File > Add Samples. g Select the file SulfamixMRM_35.d. h Click OK. | The file SulfamixMRM_35.d is on the installation disk in the \Support\Data folder. Copy this entire folder to the \MassHunter\Data\ MRM_to_DMRM folder. | | |

Task 1. Create a batch file from an existing MRM data file

| S | eps | Detaile | d Instructions | Comments | | |
|---|--|--|--|---|--|--|
| 2 | Create a method for that batch using MRM data. | a Click from b Sele file. c Click | < Method > New > New Method n Acquired MRM data. act the SulfamixMRM_35.d data < Open. | | | |
| 3 | Set the Concentration Setup, Qualifier Setup, and Calibration Curve Setup. Add calibration level 1 with a concentration of 10000. Set the Uncertainty to Relative for all qualifiers. Set the Curve Fit to Linear. Set the Curve Fit Origin to Include. Set the Curve Fit Weight to None. | a Sele Man Met b Sele c Righ click shor d Ente the 0 e Righ Cop f Clicl g Sele Setu Task h Veriti i Sele Man Met j Set 0 com k Set 0 com I Set 0 com | tect Concentration Setup in the hual Setup Tasks section in the hod Tasks pane. ect the first compound in the table. ect the first compound row and K New Calibration Level from the tcut menu. er 1 in the Level column and 10 in Conc. column. et-click in the Level box and click y Calibration Levels To. K Select All. Click OK. etc Qualifier Setup in the Manual up Tasks section in the Method is pane. fy that the Uncertainty is Relative. etc Calibration Curve Setup in the hual Setup Tasks section in the hod Tasks pane. Curve Fit to Linear for all pounds. CF Origin to Include for all pounds. CF Weight to None for all pounds. | Refer to the online Help in the Quantitative Analysis program for additional help on these tasks. | | |

Task 1. Create a batch file from an existing MRM data file

| Steps | | Detailed Instructions | Comments |
|--|--|---|--|
| Agilent MassHunter Qu File Edit View Analyz | antitative Analysis - [New Method] e Method Update Report Tools Help Analyze Batch i @ i Larvout 📆 [13] 550 FM [17] | Restore Default Layout | |
| Method Tasks • X | Method Table | 1 Greate Levels : Time Segment: de cAllo y eb C | ampound: Sei C |
| Hethod Setup Tasks MRM Compound Set. Renerion Time Setup Concentration Setup Concentration Setup Contentration Curve Set. Globals Setup Save / Exit | Sample Data File SuffarmoNFM_1SuffarmONFM_1 SuffarmONFM_1 Quantifier Image: Compound_1 Compound_1 1211.0 > 155 Compound_2 1285.0 > 155 Compound_4 1271.0 > 155 Sample Information Sample Information | Type Level Acq Method File Acq Date-Time n Scan Type CF 7 M/SM Target Linear 7 M/SM Target Linear 8 M/SM Target Linear | CF Origin CF Weight Include None Include None Include None Include Vione |
| eiji Valdobe Iiji Save Save Ac Ki Ecit Manual Setup Tasks Outher Setup Tasks Advanced Tasks | Image: Signal (* 3 T/k) Max * of panels 2 * Signal (*None> * Jill JIL*) * Tic MMM (* -> *) SubmickMM_10.d * 1 * Tic MMM (* -> *) SubmickMM_10.d * 1 * 10 1 0 2 0 3 0 4 05 0 6 07 0 8 0 9 1 1.1 12 13 14 15 16 17 18 19 2 21 22 23 24 Compaute Information * X | | |
| | • MRM (2710-> 155 8) SuffernizhRR_10.4 # x10-1 0 0- 0- 0- 0.05 01 0.15 02 0.25 0.3 0.35 | 0.445min. 04 0.45 05 0.55 0.6 0.85 07 0.75 0.8 0.85 0.9 0.85 | 105 11 115 12 125 13 135 14 Acquaision Time (mig) A Comment (Auto NTIME (Mig)) |
| 4 Verify met method ar the batch. | hod and then save the nd apply the method to | a Click Method > Validate b Click OK on the message errors, if necessary. c Click Method > Save As d Enter MRM_to_DMRN e Click the Save button. f Click Method > Exit. g Click Yes to apply the m batch. | e. e box. Fix any s. 4. ethod to the |
| 5 Analyze a | nd save the batch. | a Click Analyze > Analyze | e Batch. |

b Click File > Save Batch.

Task 2. Print a report in the Quantitative Analysis program

Task 2. Print a report in the Quantitative Analysis program

In this task, you print a report using any template.

You can update a Dynamic MRM method using either a data file or a quantitation report folder, so this task creates the quantitation report folder.

| Steps | | Detailed Instructions | Comments |
|-------|--|--|---|
| 1 | Print a report using the template MRM_to_DMRM.xltx. | a Click File > Save. b Click Report > Generate. The system displays the Report dialog box. c Select the Template file. d Select the Report folder. This folder name will be used in the next task. e Click OK. | Copy the MRM_to_DMRM.xltx template from the \Support\Data folder on the installation disk. For this report, you do not need to print the report. You need to click Advanced to select a different printer. If you don't want to print this report, click Advanced instead. |
| 2 | Check the status of the report using the Queue Viewer program. | a Click Report > Queue Viewer. b Wait for the report to finish printing. c Close the Task Queue Viewer program. | |
Exercise 2 – Develop a Dynamic MRM acquisition method from an MRM acquisition data file or an MRM method

Task 3. Create a Dynamic MRM method using Update dMRM

Task 3. Create a Dynamic MRM method using Update dMRM

You can create a Dynamic MRM method from an MRM data file or a Quantitative Analysis method. You first set the **Scan Type** to Dynamic MRM, and then you use the Update MRM Method dialog box.

| a Click File > Open > Method. b Select the <i>iii</i>Sulfamix MRM_10.m method. Click OK. c Click Method > Save As. d Type the new method name with the format <i>iii</i>Sulfamix_dMRM.m. | In this example, the batch is in the \MassHunter\Data\ MRM_to_DMRM folder. |
|--|---|
| a Click the Acquisition tab in the QQQ tab in the Method Editor window. b Right-click the Scan segments table and click Update MRM Method. The Update MRM Editor dialog box opens. c Select the folder containing the report.results.xml file or the data file iiiSulfamix MRM_10.d. d Select True for Update Retention Time?. e Select True for Add new Compound. | The Update MRM Method tool automatically sets the Scan type to Dynamic MRM. You can select either a data file that was acquired with a Scan Type of MRM or a Quant Report folder as the input to this dialog box. The Scan segments are created from one of these two input sources. |
| | a Click File > Open > Method. b Select the <i>iii</i>Sulfamix MRM_10.m method. Click OK. c Click Method > Save As. d Type the new method name with the format <i>iii</i>Sulfamix_dMRM.m. a Click the Acquisition tab in the QQQ tab in the Method Editor window. b Right-click the Scan segments table and click Update MRM Method. The Update MRM Editor dialog box opens. c Select the folder containing the <i>report.results.xml</i> file or the data file <i>iiiSulfamix MRM_10.d</i>. d Select True for Update Retention Time?. e Select True for Add new Compound. f Click OK. |

| D:\Mas | sHunter\Data\MRM_to_DMRM | | |
|--------|--------------------------------------|----------------|--|
| 🖃 Met | hod Optionsi | | |
| Add | new Compound? | True | |
| Peal | k Abundance Threshold | 50 | |
| Cyc | le Time | 500 | |
| 🗄 Ret | ention Time | | |
| Upd | ate Retention Time? | True | |
| Upd | ate Retention Time Window? | True | |
| Scal | le Factor of RT Window to Peak Width | 3 | |
| Rete | ention Time Window Threshold | 10 | |
| Rete | ention Time Window Threshold Unit | Percent | |
| 🗉 Trig | ger Threshold | | |
| Upd | ate Threshold? | False | |
| Perc | ent of Height | 10 | |
| Scal | le Factor | 1 | |
| 🗆 Trig | iger Window | | |
| Upd | ate Trigger Window? | True | |
| Trig | ger Window Options | Retention Time | |
| Abs | olute value (mins) | 0.4 | |
| Perc | cent value | 10 | |
| Scal | le Factor | 1 | |
| Metho | d Options | | |

You can update the compounds in the Scan segments table by using a QQQ data file or a Quantitative analysis report folder.

$\label{eq:exercise 2-Develop a Dynamic MRM acquisition method from an MRM acquisition data file or an MRM method$

Task 3. Create a Dynamic MRM method using Update dMRM

| teps | Detaile | ed Instru | ctio | ns | | | | Con | nme | nts | | | |
|--|--------------------|-----------------|---------|-----------------|---------|---------------|---------|-------------------|-------------------|------------|---------|----------------------------|------------|
| Tune file Stop time dourse.TUNE3ML @ No limit/As Pump | Acquisition Source | Chromatogram | strumen | t Diagnostics | 1 | | | | | | Levi | | |
| Browse 6d C 1 min | Compound Group | Compound Name / | ISTD? | Precursor Ion 7 | MS1 Res | Product Ion V | MS2 Res | Het Time (min) | Delta Ret Time | Fragmentor | Energy | Cell Accelerato Voltage | Polarity |
| Ion source | | Compound_1 | | 311 | Unit | 155.7 | Unit | 2.15 | 0.81 | 100 | 0 | 1 | 7 Positive |
| | | Compound_2 | | 205 | Unit | 155.7 | Unit | 0.74 | 0.55 | 100 | 0 | 1 | 7 Positive |
| Peak width 0.07 min | | Compound_3 | | 279 | Unit | 185.7 | Unit | 1.16 | 1.17 | 100 | 0 | 1 | Positive |
| Time segments | • | Compound_4 | | 271 | Unit | 155.8 | Unit | 0.41 | 0.37 | 100 | 0 |) 7 | Positive |
| | | | | | | | | | | | | | |
| | Dynamic MRM Parame | lers | | | | | | | | Triggere | ed MRM | | |
| cycles/s ms/cycle | Cycle Time 500 | ms | | | | | | | | E Tri | iggered | Repeats | 3 |
| | | | | | | | | | | | | | |

The compounds from the data file or quantitation report are automatically added to the Scan segments table.

| g | Select the original compound in the |
|---|--|
| | Scan segments table. |
| h | Right-click the row and click Delete |
| | Row. |
| i | Verify that each row has a Compound |
| | Name. A blank Compound Name is not |
| | allowed. |
| j | Click Method > Save. |

Exercise 2 – Develop a Dynamic MRM acquisition method from an MRM acquisition data file or an MRM method

Task 4. Create a Dynamic MRM method from an MRM method

Task 4. Create a Dynamic MRM method from an MRM method

You can create a Dynamic MRM method directly from an MRM method by using the Paste from Clipboard command from the shortcut menu.

| S | teps | Detailed Instructions | Comments | | |
|---|---|---|--|--|--|
| 1 | Open the method <i>iii</i> Sulfamix MRM_10.m and save it to a new name with the format <i>iii</i> Sulfamix dMRM_Easy.m, where <i>iii</i> are your initials. | a Click File > Open > Method. b Select the <i>iii</i>Sulfamix MRM_10.m method. c Click OK. d Click Method > Save As. e Type the new method name with the format <i>iii</i>Sulfamix_dMRM2.m. f Click the Save button. | | | |
| 2 | Copy all compounds from the Scan segments table in the MRM method. | a Click the Acquisition tab in the QQQ tab in the Method Editor. b Select all of the rows in the Scan segments table. c Right-click the Scan segments table and click Copy. | • To select all of the rows in the Scan segments table, you select the first row in the table, Then, you scroll to the last row in the Scan segments table. Press the Shift key and select the last row in the table. | | |
| 3 | Change the Scan Type to Dynamic MRM and paste the rows into the new Scan segments table. | a Select Dynamic MRM as the Scan Type. b Right-click the Scan segments table and click Paste from Clipboard. c Select the original compound in the Scan segments table. d Right-click and click Delete Row. e Click Method > Save. | • To combine multiple Time Segments into one Dynamic MRM Time Segment, you paste the Scan segments into Excel and create one long list. When all of the scan segments have been pasted into Excel, then copy all of the Scan segments in Excel. | | |

| Tune file Stop time atures.tune.xml | Acquisition Source Chromatogram Instrument Diagnostics | |
|---|---|---|
| Browse 65 C 1 min | Compound Name / ISTD? Precursor C MS1 Res Product Ion C MS2 Res Fragmentor Collision Cell Accelerator Voltage | r Ret Time Delta Ret (min) Time Polarity |
| lon source | Compound1 350 Unit 200 Unit Insert Row | 0 0 Positive |
| ESI 💌 🔽 Agilent Jet Stream 🔽 Peak width 0.07 min | sultamethoxine 311 Unit 155.7 Unit Append Row | 5 U.81 Positive |
| - Time commente | sulfachioropyradizin 285 Unit 155.7 Unit Delete Row | 4 U.55 Positive |
| # Start / Scan Tune Div Valve Delta Delta Stored | sutrametrizole 271 Unit 155.8 Unit Sort | 1 0.37 Positive |
| Imme EMV (*) EMV (*) EMV (*) I 0 Dynamic MRM To MS 0 0 Ø | Import from optimizer Update MRM Method Edit MRM Method Calibrate MRM Method | |
| cyclez/s mz/cycle | Dynamic MRM Parameters Cut Cycle Time 500 ms Parte Parte Cubeard | per of Repeats 3 |

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

Task 1. Create a Triggered Dynamic MRM method from a Dynamic MRM method manually

You can create a Triggered Dynamic MRM method directly from a Dynamic MRM method. In a Triggered Dynamic MRM method, you specify some of the transitions to be primary transitions. These transitions are acquired for the entire retention time window. Some of these primary transitions are also marked as triggers. As the data is acquired, the software checks whether or not the abundances of the trigger transitions are higher than the threshold. If the abundances are higher than the thresholds and other additional conditions are met, then the secondary transitions are acquired. These other conditions are described in the *Concepts* guide.

| Steps | Detailed Instructions | Comments | | | |
|---|---|---|--|--|--|
| Open the method <i>iii</i>Sulfamix MRM_10.m and save it to a new name with the format <i>iii</i>Sulfamix dMRM_Easy.m, where <i>iii</i> are your initials. | a Click File > Open > Method. b Select the <i>iiiSulfamix_dMRM2.m</i> method. c Click OK. d Click Method > Save As. e Type the new method name with the format <i>iiiSulfamix_TriggeredDMRM.m.</i> f Click the Save button. | A Triggered Dynamic MRM method is a type of Dynamic MRM method. The Scan Type for both methods is Dynamic MRM. The Dynamic MRM method is the template method for the optimization. | | | |

Task 1. Create a Triggered Dynamic MRM method from a Dynamic MRM method manually

| St | teps | Detailed Instructions Comments | Comments | | | |
|----|--|---|--|--|--|--|
| 2 | Change the method to a triggered dynamic MRM method. | a Click the Acquisition tab in the QQQ tab in the Method Editor. b Mark the Triggered check box in the Triggered MRM section. This section is only available if the Scan Type is Dynamic MRM. c Select whether to automatically mark the highest product ion as the Primary. d Enter the value for Repeats. Several columns are added Scan segments table. The columns only apply to a tri dynamic MRM method. The value Repeats is the n times to acquire each of the secondary transitions when triggering conditions are n to the value for Repeats. | d to the se ggered number of ne en the net. | | | |
| 3 | Select the transitions that are the Primary transitions. | a For each transition, mark the Primary check box if it is a Primary transition. b Verify that you have marked at least one transition as the Primary transition for each Compound Name. You can select multiple transition. transition. If a transition is same Compound Name, the part of the same compound must mark at least one transition for each compound. | nsitions e Primary nas the nen it is d. You nsition as ch | | | |
| 4 | Select the transitions that are the Trigger transitions and set the trigger conditions. | a For each compound, mark the Trigger check box if it is a Trigger transition. b (optional) Mark a second Trigger transition. c (optional) Enter the Threshold value for each Trigger transition. d (optional) Enter the Trigger Entrance for each Trigger transition. e (optional) Enter the Trigger Delay for each Trigger transition. f (optional) Enter the Trigger Window for each Trigger transition. f (optional) Enter the Trigger Window for each Trigger transition. For each compound, you of two Trigger transitions. For each compound, you of two Trigger transitions. If the Trigger transition has abundance over the Threshold value that triggering condition is By default, the Trigger Entrance for each Trigger Tentsition. g (optional) Enter the Trigger Delay for each Trigger transition. f (optional) Enter the Trigger Window for each Trigger transition. See the Concepts Guide for information on these trigg conditions. | an have s an hold , then s met. rance , Trigger se values g l. or more er | | | |

Task 2. Add/Modify compounds in an existing database

| Steps | | | Detailed Instructions | | | | | | Comments | | | | | | | | | |
|--|-----------------------|-----------|-----------------------|---------|----------|-----------|-------------------|-------------------|------------|---------------------|---------------------|------------------|-------------------|---------------------|------------|------------|------------|-------|
| Scan segment | r yr ramarit | | | | | | | | | | | | | | | | | |
| Compound Group | Compound Name | Precursor | Product Ion | Primary | Trigger | Threshold | Ret Time (min) | Delta Ret Time | Fragmentor | Collision Energy | Trigger Entrance | Trigger Delay | Trigger Window | Cell Accelerator | Polarity / | MS1 Res | MS2 Res | ISTD? |
| 1 | sulfachloropyridazine | 285 | 197 | • | v | 800 | 0.5 | 1 | 380 | 8 | 2 | 0 | 0 | 7 | Positive | Unit | Unit | |
| | sulfachloropyridazine | 285 | 156 | ~ | | | 0.5 | 1 | 380 | 8 | | | | 7 | Positive | Unit | Unit | |
| | sulfachloropyridazine | 285 | 108 | | Г | | | | 380 | 20 | | | | 7 | Positive | Unit | Unit | |
| 1 | sulfadimethoxine | 311.1 | 245.1 | ~ | • | 1000 | 1.2 | 1 | 380 | 12 | 0 | 1 | 0 | 7 | Positive | Unit | Unit | |
| | sulfadimethoxine | 311.1 | 173.1 | ~ | | | 1.2 | 1 | 380 | 24 | | | | 7 | Positive | Unit | Unit | |
| | sulfadimethoxine | 311.1 | 156 | | Г | | | | 380 | 16 | | | | 7 | Positive | Unit | Unit | |
| | sulfadimethoxine | 311.1 | 108 | | Г | | | | 380 | 24 | | | | 7 | Positive | Unit | Unit | |
| 2 | sulfamethazine | 279.1 | 186 | ~ | • | 900 | 0.8 | 1 | 380 | 12 | 0 | 0 | 0.4 | 7 | Positive | Unit | Unit | |
| | sulfamethazine | 279.1 | 155.9 | ~ | v | 1000 | 0.8 | 1 | 380 | 12 | 0 | 0 | 0.4 | 7 | Positive | Unit | Unit | |
| | sulfamethazine | 279.1 | 124.1 | | Г | | | | 380 | 20 | | | | 7 | Positive | Unit | Unit | |
| 2 | sulfamethazine | 279.1 | 108 | | Г | | | | 380 | 24 | | | | 7 | Positive | Unit | Unit | |
| | sulfamethizole | 271 | 253.4 | ~ | ~ | 1100 | 0.3 | 1 | 380 | 0 | 2 | 1 | 0.3 | 7 | Positive | Unit | Unit | |
| | sulfamethizole | 271 | 156 | 2 | | | 0.3 | 1 | 380 | 4 | | | | 7 | Positive | Unit | Unit | |
| 0 | sulfamethizole | 271 | 108 | | | | | | 380 | 20 | | | | 7 | Positive | Unit | Unit | |
| | | | | | | | | | | | | | | | | | | |
| Dynamic NRM Parameters Dynamic NRM Parameters Cycle Time 500 ms Pinnary Dryb : Total MRMs = 4 Min/Max Dwell = 32.21 ms/32.21 ms/ Triggered MRM Triggered MRM Triggered Repeats 3 | | | | | | | | | | | | | | | | | | |

Task 2. Add/Modify compounds in an existing database

You can also manually add compounds to a database and modify the compounds in the database. In the next task, you create a Triggered Dynamic MRM method from the compounds in the database.

| St | teps | Detailed Instructions | Comments | | | |
|----|---|--|--|--|--|--|
| 1 | Review the <i>iii</i> Sulfamix_dMRM2.m, where <i>iii</i> are your initials. | a Click File > Open > Method. b Select the iiiSulfamix_dMRM2.m method. c Click OK. d Review the parameters. | A Triggered Dynamic MRM method is a type of Dynamic MRM method. The Scan Type for both methods is Dynamic MRM. | | | |
| 2 | Start the MassHunter Optimizer software. | • Double-click the Optimizer icon. | If you are optimizing peptides, use the Optimizer for Peptides program. | | | |

Task 2. Add/Modify compounds in an existing database

| St | teps | D | etailed Instructions | Comments | | | |
|----|---|----------------------------|--|---|---|--|--|
| 3 | Set parameters on the Optimizer Setup tab. | a b c d e f | Click the Optimizer Setup tab. Click the Injection (with or without column) button. Set the CE range from 4 to 48. Set the Cell Accelerator Voltage to 7. Right-click the table and click Add Method. Select the iiiSulfamix_dMRM2.m method. | To create low mass product ions from a precursor ion near 300 m/ you need fairly high collision energies. | | | |
| 4 | Set parameters on the Precursor Ion Selection tab. | a b | Click the Precursor Ion Selection tab. Verify that +H is marked for the Positive ions (with priorities) list. | | | | |
| 5 | Set parameters on the Product Ion Selection tab. | a b c | Click the Product Ion Selection tab. Click the Mass (m/z) button under Low mass cut-off. Enter 60 for the low mass cut-off. | • | On the Product Ion Selection, you can automatically add up to 4 product ions per compound (for instance, 2 primaries and 2 secondaries). You want 8 to 10 peaks in the composite spectrum to prove that this is indicative of the compound, so you need to add at least some of the product ions manually. | | |

Task 2. Add/Modify compounds in an existing database

| Steps | Detailed Instructions | Comments | | | | |
|--|--|---|--|--|--|--|
| 6 Set parameters on the Compound Setup tab and add additional transitions. For Precursor ion 311, add the following product ions: 244.8, 229.7, 217.7, 172.9, 107.9, 91.9, 79.9, 64.8 For Precursor 285, add the following product ions: 129.9, 107.9, 91.9, 79.8, 64.8 For Precursor 279, add the following product ions: 212.8, 155.9, 123.9, 107.9, 91.9, 79.8, 64.9 For Precursor 271, add the following product ions: 177.8, 115.9, 107.9, 92, 80, 64.9 | a Click the Compound Setup tab. b Click the Import/Export > Import from Acquisition Methods command. c Select the iiiSulfamix_dMRM2.m method and click Open. d (optional) Right-click the tab and click Expand/Collapse All Rows. e Select one of the Product rows for one of the compounds. In this example, select the Product row 155.7 for Precursor 311. f Right-click the Product row and click Add Product Ion. In this example, you add 8 product ion rows. g Enter the Product in each of the product ion rows that were added. See "To determine product ions in the Qualitative Analysis program:" on page 45. | For each compound, we are going to add additional transitions. In the Qualitative Analysis program, you examine Product Ion data files which you acquired previously to determine additional transitions to add. See "Task 4. Determine product ion masses" on page 24. You can use the arrow keys to move between rows in the Product table. | | | | |
| | h Add product ions for compounds 1, 2 and 3. | | | | | |



Formula

Mass

Sample Position

This product ion scan has a precursor mass of 311. You examine the MS spectrum to determine the product ions to add to the Product ion section of the Compound Setup table.

The product ions that are manually added as additional Product ions in the MassHunter Optimizer software are shown in the MS Spectrum Results window. The green boxes were added in this guide to show which product ions were used.

64.8

sulfamethazine

Compound Name

Groups

.

Task 2. Add/Modify compounds in an existing database

| St | teps | D | etailed Instructions | C | Comments | | | | |
|----|---|----------------------------|--|---|---|--|--|--|--|
| 3 | Set parameters on the Optimizer Setup tab. | a b c d e f | Click the Optimizer Setup tab. Click the Injection (with or without column) button. Set the CE range from 4 to 48. Set the Cell Accelerator Voltage to 7. Right-click the table and click Add Method. Select the iiiSulfamix_dMRM2.m method. | • | To create low mass product ions from a precursor ion near 300 <i>m/z</i> , you need fairly high collision energies. | | | | |
| 4 | Set parameters on the Precursor Ion Selection tab. | a b | Click the Precursor Ion Selection tab. Verify that +H is marked for the Positive ions (with priorities) list. | | | | | | |
| 5 | Set parameters on the Product Ion Selection tab. | a b c | Click the Product Ion Selection tab. Click the Mass (m/z) button under Low mass cut-off. Enter 60 for the low mass cut-off. | • | On the Product Ion Selection, you can automatically add up to 4 product ions per compound (for instance, 2 primaries and 2 secondaries). You want 8 to 10 peaks in the composite spectrum to prove that this is indicative of the compound, so you need to add at least some of the product ions manually. | | | | |

Task 2. Add/Modify compounds in an existing database

| Steps | Detailed Instructions | Comments |
|--|--|---|
| 6 Set parameters on the Compound Setup tab and add additional transitions. For Precursor ion 311, add the following product ions: 244.8, 229.7, 217.7, 172.9, 107.9, 91.9, 79.9, 64.8 For Precursor 285, add the following product ions: 129.9, 107.9, 91.9, 79.8, 64.8 For Precursor 279, add the following product ions: 212.8, 155.9, 123.9, 107.9, 91.9, 79.8, 64.9 For Precursor 271, add the following product ions: 177.8, 115.9, 107.9, 92, 80, 64.9 | a Click the Compound Setup tab. b Click the Import/Export > Import from Acquisition Methods command. c Select the iiiSulfamix_dMRM2.m method and click Open. d (optional) Right-click the tab and click Expand/Collapse All Rows. e Select one of the Product rows for one of the compounds. In this example, select the Product row 155.7 for Precursor 311. f Right-click the Product row and click Add Product Ion. In this example, you add 8 product ion rows. g Enter the Product in each of the product ion rows that were added. See "To determine product ions in the Qualitative Analysis program:" on page 45. | For each compound, we are going to add additional transitions. In the Qualitative Analysis program, you examine Product Ion data files which you acquired previously to determine additional transitions to add. See "Task 4. Determine product ion masses" on page 24. You can use the arrow keys to move between rows in the Product table. |
| | h Add product ions for compounds 1, 2 and 3. | |



Formula

Mass

Sample Position

This product ion scan has a precursor mass of 311. You examine the MS spectrum to determine the product ions to add to the Product ion section of the Compound Setup table.

The product ions that are manually added as additional Product ions in the MassHunter Optimizer software are shown in the MS Spectrum Results window. The green boxes were added in this guide to show which product ions were used.

217.7

172.9 107.9

91.9

79.9 64.8

sulfamethazine

Compound Name

Groups

.

Task 2. Add/Modify compounds in an existing database

| Steps | Detailed Instructions | Comments | | | |
|--|---|--|--|--|--|
| To determine product ions in the Qualitative Analysis program: | a Open the SulfamixPl_15.d from "Task 4. Determine product ion masses" on page 24. b Click Find > Find Compounds by Targeted MS/MS. c Close the Compound List window. d Select a compound in the Data Navigator window. For this example, click Cpd 4. e Click the Autoscale Y-axis in the MS Spectrum Results toolbar. f Right-click and drag to zoom in on the MS canatum | If possible, rearrange the windows on the screen so you can see the Optimizer program and the Qualitative Analysis program at the same time. | | | |



This product ion scan has a precursor mass of 311. You examine the MS spectrum to determine the product ions to add to the Product ion section of the **Compound Setup table.**

- 7 Set other parameters in the Compound Setup tab and start the optimization.
 - You cannot perform a multi-compound run.
 - table to use.
- a Mark the check box in the left column at the top of the table. The check box for every row in the table is marked.
- **b** Clear the **Perform multi-compound** run check box in the right column.
- You have to mark each row in the **c** Click the **Start Optimization** button in the Optimizer toolbar.

You cannot perform a multi-compound run with the number of transitions that were added. If you mark this check box. then the Expected peak width (base) is automatically set to almost 80 seconds wide. If you clear this check box, then the Expected peak width is calculated to be around 9 seconds which is more appropriate.

Task 2. Add/Modify compounds in an existing database

| eps | De | tailed Instructions | Comments |
|--|--|---|--|
| Examine the Optimize | Report. a | Examine the Collision I Product Ion. Print or save the report | Energy for each |
| | Optimizer Report | | |
| Project Name: Instrument Name: Satyr Instrument Model: 06490A Compound Name Formula | Mass Sample Position | | As a general rule, as the Product lons get smalle the optimal Collision Energy gets larger. Howeve |
| Method Name D:\MassHunter\Methods\FamGuide\03_ | a 03_MRM_to_dMRM.m | Polarity Ion Source Positive AJS ESI | when you also examine the abundance, you can see that if the Collision Energy is set to 48 for the |
| Precursor Ion Fragmentor 265 380 | Product Ion Collision Energy 155.7 10 122.9 24 107.9 24 91.9 24 64.8 48 sss Sample Position | gy Abundance 57265/4 315460 5015922 748199 1520348 6508111 | smallest product ion, the smallest product ion ca become the dominant peak. The collision energie are further adjusted later in this task. |
| Suiradimethoxine Method Name D:\MassHunter\Methods\FamGuide\D3_ | 1 03_MRM_to_dMRM.m | Polarity Ion Source Positive AJS ESI | |
| Precursor Ion Fragmentor 311 380 311 380 311 380 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 311 300 | Product Ion Collision Ener 155.7 15 244.8 12 228.7 20 217.7 16 107.9 24 91.9 32 91.9 42 64.8 43 845 58 | gy Abundance 335553 549401 135778 439405 439405 2291401 3102465 761344 2500606 | |
| sulfamethazine Method Name D.:Massi-kunter (Methods)FamGuide\03 | 1 03 MRM to dMRM.m | Polarity Ion Source Positive AJS ESI | |
| Precursor Ion Fragmentor 279 380 270 380 270 380 270 380 270 380 270 380 270 380 270 380 270 380 | Product Ion Collision Ener 185.7 11 212.8 20 155.9 12 122.9 24 107.9 24 91.9 24 64.9 48 64.9 55 55 Sample Position | 07 Aburdance 370036 41592 127752 262040 240737 3645700 124201 3798052 | |

| 9 Save the compounds. | Click the File > Save Compounds command. | |
|--------------------------------------|---|--|
| 10 Import compounds from a database. | Click the Import/Export > Import from • Database command. The Database Browser program opens. | You can also import compounds that were distributed as part of a database. |

Polarity Ion Source

Printed on: 9/12/2012 at 11:21 AM

Page 1 of 2

Method Name

K Agilent Technologies

Task 2. Add/Modify compounds in an existing database

| Steps | Detailed Instructions | Comments | | | | |
|--|--|---|--|--|--|--|
| 11 In the Database Browser program, select the transitions. | a Mark the Show All Records check box. b Click the Select top button under Select Transitions. c Type 10 for the ranked transitions. d Click the Select Transitions button. | All the transitions that you typed in are visible. The tools to allow you to set up Primary transitions and Secondary transitions are available in this program. | | | | |

| Select Transitions Select top 10 ranked transitions Primary transitions Select Transitions Secondary transitions Select Transitions | | | | | | Set primary and trigger flags Set top 2 ranked transitions as primary Set Primaries and Trigger | | | | | s by e Factor | | | |
|---|---------|----|----------|---------|-----------|---|------|----|-----|---------|---------------------|------|-----------|-----------|
| Compound Name | Formula | MW | Polarity | Species | Precursor | Product | Frag | CE | CAV | Primary | Trigger | RT | RT Window | Abundance |
| 9 📝 sulfachloropyridazin | | | Positive | | 285 | 155.7 | 380 | 10 | 3 | 7 🔽 | | 0.65 | 1 | 5723674 |
| 🥒 🗹 sulfachloropyridazin | | | Positive | | 285 | 129.9 | 380 | 24 | | 7 🗸 | | 0.65 | 1 | 315469 |
| 🖉 🗹 sulfachloropyridazin | | | Positive | | 285 | 107.9 | 380 | 24 | 3 | 7 🔽 | | 0.65 | 1 | 5015922 |
| 🦸 🔽 sulfachloropyridazin | | | Positive | | 285 | 91.9 | 380 | 24 | 1 | 7 🔽 | | 0.65 | 1 | 7438199 |
| 🖉 🗹 sulfachloropyridazin | | | Positive | | 285 | 79.8 | 380 | 48 | | 7 🔽 | | 0.65 | 1 | 1529348 |
| 🥒 💟 sulfachloropyridazin | | | Positive | | 285 | 64.8 | 380 | 48 | 7 | 7 🔽 | | 0.65 | 1 | 6508111 |
| 🥒 🗹 sulfadimethoxine | | | Positive | | 311 | 155.7 | 380 | 15 | 1 | 7 🔽 | | 2.03 | 1 | 3335528 |
| 🖉 🔽 sulfadimethoxine | | | Positive | | 311 | 244.8 | 380 | 12 | 3 | 7 🔽 | | 2.03 | 1 | 548481 |
| 1 vitadimethoxine | | | Positive | | 311 | 229.7 | 380 | 20 | 3 | 7 🔽 | | 2.03 | 1 | 135578 |
| 🥒 🔽 sulfadimethoxine | | | Positive | | 311 | 217.7 | 380 | 16 | 3 | 7 🔽 | | 2.03 | 1 | 434965 |
| 🖉 🗹 sulfadimethoxine | | | Positive | | 311 | 172.9 | 380 | 24 | 3 | 7 🔽 | | 2.03 | 1 | 414589 |
| 🥒 🔽 sulfadimethoxine | | | Positive | | 311 | 107.9 | 380 | 20 | 1 | 7 🔽 | | 2.03 | 1 | 2291401 |
| 1 sulfadimethoxine | | | Positive | | 311 | 91.9 | 380 | 32 | 3 | 7 🔽 | | 2.03 | 1 | 3102685 |
| 🥒 🗹 sulfadimethoxine | | | Positive | | 311 | 79.9 | 380 | 48 | | 7 🗸 | | 2.03 | 1 | 761344 |
| 🥒 🗹 sulfadimethoxine | | | Positive | | 311 | 64.8 | 380 | 48 | (| 7 🔽 | | 2.03 | 1 | 2583686 |
| 🖉 🗹 sulfamethazine | | | Positive | | 279 | 185.7 | 380 | 11 | 3 | 7 🔽 | | 0.98 | 1 | 3720936 |
| 🖉 🗹 sulfamethazine | | | Positive | | 279 | 212.8 | 380 | 20 | 5 | 7 🔽 | | 0.98 | 1 | 421592 |
| 🥒 🗹 sulfamethazine | | | Positive | | 279 | 155.9 | 380 | 12 | Ĵ. | 7 🔽 | | 0.98 | 1 | 1377529 |
| 🖉 🗹 sulfamethazine | | | Positive | | 279 | 123.9 | 380 | 24 | | 7 | | 0.98 | 1 | 2633848 |
| 🥒 🔽 sulfamethazine | | | Positive | | 279 | 107.9 | 380 | 24 | 5 | 7 🔽 | | 0.98 | 1 | 2407737 |
| 🖉 🗹 sulfamethazine | | | Positive | | 279 | 91.9 | 380 | 24 | į | 7 🔽 | | 0.98 | 1 | 3643700 |
| 🥒 🔽 sulfamethazine | | | Positive | | 279 | 79.8 | 380 | 48 | 1 | 7 🔽 | | 0.98 | :1 | 1242301 |
| 🖉 🗹 sulfamethazine | | | Positive | | 279 | 64.9 | 380 | 48 | 5 | 7 🔽 | | 0.98 | 1 | 3793552 |
| 🖉 🔽 sulfamethizole | | | Positive | | 271 | 155.8 | 380 | 6 | 3 | 7 🔽 | | 0.37 | 1 | 4578854 |
| 1 sulfamethizole | | | Positive | | 271 | 177.8 | 380 | 12 | | 7 🔽 | | 0.37 | 1 | 89336 |
| 🖉 🔽 sulfamethizole | | | Positive | | 271 | 115.9 | 380 | 16 | 3 | 7 🔽 | | 0.37 | 1 | 515222 |
| 🖉 🗹 sulfamethizole | | | Positive | | 271 | 107.9 | 380 | 24 | 3 | 7 🔽 | | 0.37 | 1 | 3108252 |
| 1 🔽 sulfamethizole | | | Positive | | 271 | 92 | 380 | 28 | 1 | 7 🗸 | | 0.37 | 1 | 5192801 |

12 In the Database Browser program, automatically select the **Primary** transitions and **Trigger** transition.

- a In the Set top ranked transitions as primary box, enter 2.
- **b** Click the **Set Primaries and Trigger** button.
- The software automatically selects the two most abundant transitions as the Primary transitions.
- The software also selects the most abundant transition as the **Trigger**.
- You can manually select a second **Trigger** transition.

Task 2. Add/Modify compounds in an existing database

| Steps | | | | | Detailed Instructions | | | | | | | Comments | | | | | | | |
|--|----------------------|---------|----|-------------|-----------------------|-----------|---|------|----|-----|--|-------------|------|-----------|-----------|--|--|--|--|
| Select Transitions Select top 10 ranked transitions Primay transitions Secondary transitions | | | | Select Tran | nsitions | Se | Set primary and trigger flags Set top 2 ranked transitions as primary Set Primaries and Trigger | | | | nk transitions Abundanc Response | e Factor | | | | | | | |
| | Compound Name | Formula | MW | Polarity | Species | Precursor | Product | Frag | CE | CAV | Primary | Trigger | BT | RT Window | Abundance | | | | |
| 20 | sulfachloropyridazin | | | Positive | | 285 | 155.7 | 380 | 10 | 7 | | | 0.65 | 1 | 5723674 | | | | |
| 0 1 | sulfachloropyridazin | | | Positive | | 285 | 129.9 | 380 | 24 | 7 | | | 0.65 | 1 | 315469 | | | | |
| 1 🗸 | sulfachloropyridazin | | | Positive | | 285 | 107.9 | 380 | 24 | 7 | | | 0.65 | 1 | 5015922 | | | | |
| 12 | sulfachloropyridazin | | | Positive | | 285 | 91.9 | 380 | 24 | 7 | V | V | 0.65 | 1 | 7438199 | | | | |
| 1 🔽 | sulfachloropyridazin | | | Positive | | 285 | 79.8 | 380 | 48 | 7 | | 15 | 0.65 | 1 | 1529348 | | | | |
| 1 1 | sulfachloropyridazin | | | Positive | | 285 | 64.8 | 380 | 48 | 7 | V | | 0.65 | 1 | 6508111 | | | | |
| 1 🔽 | sulfadimethoxine | | | Positive | | 311 | 155.7 | 380 | 15 | 7 | 7 | V | 2.03 | 1 | 3335528 | | | | |
| 1 🔽 | sulfadimethoxine | | | Positive | | 311 | 244.8 | 380 | 12 | 7 | 1 11 | | 2.03 | 1 | 548481 | | | | |
| 1 🗹 | sulfadimethoxine | | | Positive | | 311 | 229.7 | 380 | 20 | 7 | | | 2.03 | 1 | 135578 | | | | |
| 1 🔽 | sulfadimethoxine | | | Positive | | 311 | 217.7 | 380 | 16 | 7 | | | 2.03 | 1 | 434965 | | | | |
| 1 🔽 | sulfadimethoxine | | | Positive | | 311 | 172.9 | 380 | 24 | 7 | | | 2.03 | 1 | 414589 | | | | |
| 1 | sulfadimethoxine | | | Positive | | 311 | 107.9 | 380 | 20 | 7 | | | 2.03 | 1 | 2291401 | | | | |
| 1 🔽 | sulfadimethoxine | | | Positive | | 311 | 91.9 | 380 | 32 | 7 | V | | 2.03 | 1 | 3102685 | | | | |
| 1 🗹 | sulfadimethoxine | | | Positive | | 311 | 79.9 | 380 | 48 | 7 | | | 2.03 | 1 | 761344 | | | | |
| 1 | sulfadimethoxine | | | Positive | | 311 | 64.8 | 380 | 48 | 7 | | | 2.03 | 1 | 2583686 | | | | |
| .1 🔽 | sulfamethazine | | | Positive | | 279 | 185.7 | 380 | 11 | 7 | 1 | | 0.98 | 1 | 3720936 | | | | |
| .1 💌 | sulfamethazine | | | Positive | | 279 | 212.8 | 380 | 20 | 7 | | | 0.98 | 1 | 421592 | | | | |
| | sulfamethazine | | | Positive | | 279 | 155.9 | 380 | 12 | 7 | | | 0.98 | 1 | 1377529 | | | | |
| .1 🔽 | sulfamethazine | | | Positive | | 279 | 123.9 | 380 | 24 | 7 | | | 0.98 | 1 | 2633848 | | | | |
| 1 🔽 | sulfamethazine | | | Positive | | 279 | 107.9 | 380 | 24 | 7 | | | 0.98 | 1 | 2407737 | | | | |
| I 🗹 | sulfamethazine | | | Positive | | 279 | 91.9 | 380 | 24 | 7 | | | 0.98 | 1 | 3643700 | | | | |
| 1 🗹 | sulfamethazine | | | Positive | | 279 | 79.8 | 380 | 48 | 7 | | | 0.98 | 1 | 1242301 | | | | |
| .1 🔽 | sulfamethazine | | | Positive | | 279 | 64.9 | 380 | 48 | 7 | 7 | V | 0.98 | 1 | 3793552 | | | | |
| 1 🗹 | sulfamethizole | | | Positive | | 271 | 155.8 | 380 | 6 | 7 | | | 0.37 | 1 | 4578854 | | | | |
| 1 | sulfamethizole | | | Positive | | 271 | 177.8 | 380 | 12 | 7 | | | 0.37 | 1 | 89336 | | | | |
| 1 | sulfamethizole | | | Positive | | 271 | 115.9 | 380 | 16 | 7 | | | 0.37 | 1 | 515222 | | | | |
| .1 🔽 | sulfamethizole | | | Positive | | 271 | 107.9 | 380 | 24 | 7 | | | 0.37 | 1 | 3108252 | | | | |
| .0 🗸 | sulfamethizole | | | Positive | | 271 | 92 | 380 | 28 | 7 | 7 | V | 0.37 | 1 | 5192801 | | | | |

You examine the Primary column and the Trigger column to determine which transitions are selected. You can select one or two Trigger transitions. You can select multiple Primary transitions.

- **13** Review the Primary transitions and Trigger transitions.
 - For sulfachloropyridazine, select 285 m/z -> 156 m/z transition as
 the Primary and Trigger transition.
 - For sulfadimethoxine, select select 311 m/z -> 156 m/z transition as the Primary and Trigger transition.
 - For sulfamethazine, select 279 m/z -> 186 m/z transition as the Primary and Trigger transition.
 - For sulfamethizole, select 271 m/z -> 156 m/z transition as the Primary and Trigger transition.

- Review each compound. Change the Primary and Trigger transitions to the transitions listed in the left column.
 - Change the other Primary transitions as shown below.
- The software selected the most abundant transitions which in this example often had a low m/z for the Product Ion. A very abundant low m/z ion may be unsuitable as a Primary transition.
- You can select two Primary transitions as triggers for a compound.

Task 2. Add/Modify compounds in an existing database

| Steps | | | [|)etailed | Com | nment | S | | | | | | | |
|---|-------------------|-----|-------------|----------|-----------|--|--|------------------|---------|--|---------------------|------|-----------|-----------|
| Select Transitions Select top 10 Primary transitions Secondary transitions | ranked transition | \$ | Select Tran | sitions | Se | t primary and trigge Set top 2 Set Prima | r flags ranked transi ries and Trigger | tions as primary | Ra @ | nk transitions Abundanc Response | s by e Factor | | | |
| Compound Name | Formula | MW/ | Polarity | Species | Precursor | Product | Frag | CE | CAV | Primary | Trigger | RT | RT Window | Abundance |
| sulfachloropyridazin | | | Positive | | 285 | 155.7 | 380 | 10 | 7 | | | 0.65 | 1 | 572367 |
| 🗹 sulfachloropyridazin | | | Positive | | 285 | 129.9 | 380 | 24 | 7 | | | 0.65 | 1 | 31546 |
| sulfachloropyridazin | | | Positive | | 285 | 107.9 | 380 | 24 | 7 | 1 | | 0.65 | 1 | 501592 |
| 🔽 sulfachloropyridazin | | | Positive | | 285 | 91.9 | 380 | 24 | 7 | | | 0.65 | 1 | 743819 |
| 🗹 sulfachloropyridazin | | | Positive | | 285 | 79.8 | 380 | 48 | 7 | 100 | | 0.65 | 1 | 152934 |
| sulfachloropyridazin | | | Positive | | 285 | 64.8 | 380 | 48 | 7 | | | 0.65 | 1 | 650811 |
| Sulfadimethoxine | | | Positive | | 311 | 155.7 | 380 | 15 | 7 | 9 | V | 2.03 | 1 | 33355. |
| 🔽 sulfadimethoxine | | | Positive | | 311 | 244.8 | 380 | 12 | 7 | | | 2.03 | 1 | 5484 |
| sulfadimethoxine | | | Positive | | 311 | 229.7 | 380 | 20 | 7 | | | 2.03 | 1 | 1355 |
| sulfadimethoxine | | | Positive | | 311 | 217.7 | 380 | 16 | 7 | | | 2.03 | 1 | 43496 |
| sulfadimethoxine | | | Positive | | 311 | 172.9 | 380 | 24 | 7 | | | 2.03 | 1 | 41458 |
| sultadimethoxine | | | Positive | | 311 | 107.9 | 380 | 20 | 7 | | | 2.03 | 1 | 229140 |
| sulfadimethoxine | | | Positive | | 311 | 91.9 | 380 | 32 | 7 | | | 2.03 | 1 | 310268 |
| sulfadimethoxine | | | Positive | | 311 | 79.9 | 380 | 48 | 7 | | | 2.03 | 1 | 7613 |
| Sulfadimethoxine | | | Positive | | 311 | 64.8 | 380 | 48 | 7 | | | 2.03 | 1 | 25836 |
| ✓ sulfamethazine | | | Positive | | 279 | 185.7 | 380 | 11 | 7 | | V | 0.98 | 1 | 372093 |
| sulfamethazine | | | Positive | | 279 | 212.8 | 380 | 20 | 7 | | | 0.98 | 1 | 42155 |
| sulfamethazine | | | Positive | | 279 | 155.9 | 380 | 12 | 7 | | | 0.98 | 1 | 137752 |
| sulfamethazine | | | Positive | | 279 | 123.9 | 380 | 24 | 7 | 2 | | 0.98 | 1 | 263384 |
| sulfamethazine | | | Positive | | 279 | 107.9 | 380 | 24 | 7 | | | 0.98 | 1 | 240773 |
| sulfamethazine | | | Positive | | 279 | 91.9 | 380 | 24 | 7 | | | 0.98 | 1 | 36437 |
| 🗹 sulfamethazine | | | Positive | | 279 | 79.8 | 380 | 48 | 7 | | | 0.98 | 1 | 12423 |
| sulfamethazine | | | Positive | | 279 | 64.9 | 380 | 48 | 7 | | | 0.98 | 1 | 37935 |
| sulfamethizole | | | Positive | | 271 | 155.8 | 380 | 6 | 7 | | | 0.37 | 1 | 45788 |
| sulfamethizole | | | Positive | | 271 | 177.8 | 380 | 12 | 7 | | | 0.37 | 1 | 893 |
| V sulfamethizole | | | Positive | | 271 | 115.9 | 380 | 16 | 7 | | | 0.37 | 1 | 5152 |
| 🗹 sulfamethizole | | | Positive | | 271 | 107.9 | 380 | 24 | 7 | | | 0.37 | 1 | 31082 |
| sultamethizole | | | Positive | | 271 | 92 | 380 | 28 | 7 | V | V | 0.37 | 1 | 51928 |

14 Review the Import List table on the Import List tab.

- a Click the Add to Import List button.
- **b** Click the **Import List** tab.
- c Review the Import List table.
- d Click the Import button.

 In this example, you are importing from the database to the Import List. Then, you are importing from Database Browser to Optimizer.

Task 2. Add/Modify compounds in an existing database

| teps | | | | | Detaile | d Inst | ructio | ıs | | Detailed Instructions | | | | | | | | |
|----------------------|----------|------|----------|---------|----------|---------|--------|-----|---------|-----------------------|------|------------|-------------|-----|-----------------|----------------|--------|--|
| | | | | | | | | | | | | | | | | | | |
| Concernent Name | Formula | Mbu/ | Polarito | Snacias | Pressent | Product | Eran | CF. | Primary | Trimer | PT | BT Western | Alexanderse | RF. | Acc Method | Project Name | firm | |
| sullachioropyridazin | - unital | | Postve | abecoda | 285 | 155.7 | 380 | 10 | 125 | 101 | 0.65 | 1 | 5723674 | | D:MassHunterM | DefaultProject | - Pres | |
| sullachloropundazin | | | Postve | | 285 | 129.9 | 380 | 24 | 221 | 101 | 0.65 | 1 | 315469 | | D: MassHunter M | DefaultProject | | |
| sullachloropyridazin | | | Postve | | 285 | 107.9 | 380 | 24 | 123 | 121 | 0.65 | 1 | 5015922 | | D: MassHunter M | DefaultProject | | |
| sullachloropyridazin | | | Positive | | 285 | 91.9 | 380 | 24 | 10 | 11 | 0.65 | 1 | 7438199 | | D: MassHunter M | DefaultProject | | |
| sulfachioropyridazin | | | Positive | | 285 | 79.8 | 380 | 48 | 15 | 10 | 0.65 | 1 | 1529348 | | D: MassHunter M | DefaultProject | | |
| sultachloropyridazin | | | Positive | | 285 | 64.9 | 380 | 40 | 100 | ET. | 0.65 | 1 | 6500111 | | D: MassHuwer M | DefaultProject | | |
| sultadmethoxine | | | Positive | - | 211 | 155.7 | 300 | 15 | 12 | 1 | 2.00 | 1 | 3335528 | | D: MassHunter M | DefaultProject | | |
| sulladmethoxine | | | Positive | | 311 | 244.0 | 380 | 12 | 175 | E | 2.03 | 1 | 548481 | | D: MassHunter M | DefaultProject | | |
| sulladmethoxine | | | Positive | | 311 | 229.7 | 380 | 20 | 175 | 173 | 2.03 | 1 | 135578 | | D:\MassHunter\M | DefaultProject | | |
| sulladmethoxine | | | Positive | | 311 | 217.7 | 380 | 16 | | 171 | 2.03 | 1 | 434965 | | D: MassHunter M | DefaultProject | | |
| sulladmethosine | | | Postive | | 311 | 172.9 | 390 | 24 | 17 | 12 | 2.03 | 1 | 414589 | | D: MassHunter M | DefaultProject | | |
| sulladmethosine | | | Positive | | 311 | 107.9 | 380 | 20 | 191 | 17 | 2.03 | 1 | 2291401 | | D: MassHunter M | DefaultProject | | |
| suitadimethoxine | | | Positive | | 311 | 91.9 | 390 | 32 | E3 | 11 | 2.03 | 1 | 3102685 | | D: MassHunter M | DefaultProject | | |
| sulladmethoxine | | | Positive | | 311 | 79.9 | 300 | 49 | 175 | 171 | 2.03 | 1 | 761344 | | D: MassHunter M | DefaultProject | | |
| sulladmethoxine | | | Positive | | 311 | 64.9 | 300 | 48 | E1 | 121 | 2.03 | 1 | 2583686 | | D: MassHunter M | DefaultProject | | |
| sultamethazine | | | Positive | | 279 | 105.7 | 300 | 11 | 12 | V | 0.90 | 1 | 3720936 | | D: WassHunter M | DefaultProject | | |
| sulfamethazine | | | Positive | | 279 | 212.8 | 380 | 20 | 13 | 10 | 0.98 | 1 | 421592 | | D: MassHunter M | DefaultProject | | |
| sultamethazine | | | Postive | | 279 | 155.9 | 380 | 12 | 101 | 171 | 0.98 | 1 | 1377529 | | D:MassHurterM | DefaultProject | | |
| sullamethacne | | | Postive | | 279 | 123.9 | 390 | 24 | 12 | 171 | 0.98 | 1 | 2633848 | | D: MassHunter M | DefaultProject | | |
| sulfamethazine | | | Positive | | 279 | 107,9 | 380 | 24 | (73 | 171 | 0.98 | 1 | 2407737 | | D: MassHunter M | DefaultProject | | |
| sullamethazine | | | Positive | | 279 | 91.9 | 380 | 24 | 63 | 173 | 0.98 | 1 | 3643700 | | D: MaccHunter M | DefaultProject | | |
| sulfamethazine | | | Positive | | 279 | 79.8 | 380 | 48 | 191 | 11 | 0.98 | 1 | 1242301 | | D MassHunter M | DefaultProject | | |
| sulfamethazine | | | Positive | | 279 | 64.9 | 300 | 40 | 10 | 100 | 0.90 | 1 | 3793552 | | D: MassHunter M | DefaultProject | | |
| sultamethizole | | | Positive | | 271 | 155.0 | 300 | G | (9) | 1 | 0.37 | 1 | 4578054 | | D: WassHunter M | DefaultProject | | |
| suitamethizole | | | Postve | | 271 | 177.0 | 300 | 12 | 81 | 111 | 0.37 | 1 | 89336 | | D: MassHunter M | DefaultProject | | |
| sultamethizole | | | Postve | | 271 | 115.9 | 380 | 16 | [2] | Red . | 0.37 | 1 | 515222 | | D: MassHunter M | DefaultProject | | |
| sullamethizole | | | Postve | | 271 | 107.9 | 380 | 24 | 87 | 111 | 0.37 | 1 | 3108252 | | D: WassHunler M | DefaultProject | | |
| sultamethicole | | | Positive | | 271 | 92 | 380 | 28 | [92] | 10 | 0.37 | 1 | 5192801 | | D. MassHunter M | DefaulProject | | |
| sullamethizole | | | Positive | | 271 | 80 | 380 | 48 | 63 | | 0.37 | 1 | 1196598 | | D: MassHunter M | DefaultProject | | |
| sultamethizole | | | Positive | | 271 | 64.9 | 380 | 48 | F1 | 15 | 0.37 | 1 | 4791359 | | D: WassHunter M | DefaultProject | | |

- **15** Review the Compound Setup table in Optimizer. You replace all compounds with the compounds from the Database Browser program.
- a Click the Yes to All button.
- **b** In the Compound Setup tab in the Optimizer program, review the compounds.
- The compounds in the Optimizer program are overwritten by the compounds that you updated in the Database Browser program.

| Replace | | | | | | | |
|--|--|--|--|--|--|--|--|
| Similar record for the compound 'sulfachloropyridazine' is already present in current project. | | | | | | | |
| Do you want to replace it? | | | | | | | |
| Yes Yes to All No No to All | | | | | | | |

Task 2. Add/Modify compounds in an existing database

| Steps | Detailed Instructions | Comments | | | | |
|---|--|--|--|--|--|--|
| 16 Save the new compound parameters to the database. | Click the File > Save Compounds command to save all of the changes to the database. | You cannot see these results by default, but the Primary and Trigger transitions are updated in the project. The Primary column, Trigger column, Trigger Entrance Delay column, Trigger Delay column, Trigger Window column and Trigger MRM Threshold column are available in the Compound Setup tab, but they are hidden by default. | | | | |

Task 3. Create a Triggered Dynamic MRM method from an existing database

Task 3. Create a Triggered Dynamic MRM method from an existing database

You can create a Triggered Dynamic MRM method from a database such as the Pesticides or Forensics/Tox database. These databases can be purchased from Agilent. You can also copy the information from an Excel spreadsheet, but that method is not described in this guide.

| Steps Detai | iled Instructions | Comments |
|--|--|---|
| 1 In the Data Acquisition program, you now import the updated compounds from the database. These compounds have optimized collision energies and also Primary and Trigger transitions marked. 6 In table of the table of the table of the table of tab | witch to the Data Acquisition ogram. ben the iiiSulfamix_dMRM2.m ethod. the QOQ tab, click the Acquisition b. The Scan segments table contains ur rows which are deleted later. ght-click the Scan Segments table ad click Import from Database rowser . The Database Browser ogram opens. ark the Show All Records check bx. ark all of the transitions for the four ulfa drug compounds. Clear the check bxes next to any unwanted ompounds. ick the Add to Import List button. ick the Import List table. ick the Import List table. ick the Import button . elete the original compounds from e Scan segments table. ark the Triggered check box under iggered MRM. | Before you import compounds from Database Browser, the Scan segments table contains at least one row. After importing compounds from the Database Browser, you need to remove any original rows. The Scan segments table always has to have at least one row. The triggering information is loaded from the Database Browser program even if the Triggered check box is clear. See the online Help for the Data Acquisition program and the QQQ Concepts Guide for an explanation of the other triggering conditions: Trigger Entrance, Trigger Delay, and Trigger Window. |

Task 3. Create a Triggered Dynamic MRM method from an existing database

| Steps | | | Detail | ed In | struc | tions | | | | | Com | ments | | | | |
|---------------------------------------|-----------------------------|----------|-----------------|-----------|------------------|-------------|----------|---------|-------------------|-------------------|-------------------|------------|---------------------|-----------------------------|----------|----------|
| cquisition Source | Chromatogram Ins | strument | Diagnostics | 1 | | | | | | | | | | | | |
| Compound Group | Compound Name / | ISTD? | Precursor Ion ∇ | MS1 Res | Product Ion V | MS2 Res | Primary | Trigger | Threshold | Ret Time (min) | Delta Ret Time | Fragmentor | Collision Energy | Cell Accelerator Voltage | Polarity | Trigge _ |
| • | sulfachloropyridazin | | 285 | Unit | 155.7 | Unit | | | 48875 | 0.65 | 1 | 380 | 10 | 7 | Positive | |
| | sulfachloropyridazin | | 285 | Unit | 129.9 | Unit | | | | | | 380 | 24 | 7 | Positive | |
| | sulfachloropyridazin | | 285 | Unit | 107.9 | Unit | ~ | | | 0.65 | 1 | 380 | 24 | 7 | Positive | |
| | sulfachloropyridazin | | 285 | Unit | 91.9 | Unit | | | | | | 380 | 24 | 7 | Positive | |
| | sulfachloropyridazin | | 285 | Unit | 79.8 | Unit | | | | | | 380 | 48 | 7 | Positive | |
| | sulfachloropyridazin | | 285 | Unit | 64.8 | Unit | | Г | | | | 380 | 48 | 7 | Positive | |
| | sulfadimethoxine | | 311 | Unit | 155.7 | Unit | • | 7 | 169666 | 2.03 | 1 | 380 | 15 | 7 | Positive | |
| | sulfadimethoxine | | 311 | Unit | 244.8 | Unit | | Г | | | | 380 | 12 | 7 | Positive | |
| | sulfadimethoxine | | 311 | Unit | 229.7 | Unit | | Г | | | | 380 | 20 | 7 | Positive | |
| | sulfadimethoxine | | 311 | Unit | 217.7 | Unit | | Г | | | | 380 | 16 | 7 | Positive | |
| | sulfadimethoxine | | 311 | Unit | 172.9 | Unit | | Г | | | | 380 | 24 | 7 | Positive | |
| | sulfadimethoxine | | 311 | Unit | 107.9 | Unit | V | | | 2.03 | 1 | 380 | 20 | 7 | Positive | |
| | sulfadimethoxine | | 311 | Unit | 91.9 | Unit | | Г | | | | 380 | 32 | 7 | Positive | |
| | sulfadimethoxine | | 311 | Unit | 79.9 | Unit | | Г | | | | 380 | 48 | 7 | Positive | |
| | sulfadimethoxine | | 311 | Unit | 64.8 | Unit | П | Г | | | | 380 | 48 | 7 | Positive | • |
| Dynamic MRM Paramet Cycle Time 200 | ers Total MRMs = 4 ms | Max C | ioncurrent MRMs | = 3 Min/N | 1ax Dwell = 65.7 | 8 ms/199.20 |) ms | | Triggered Trig | I MRM | Repe | ats 3 | | | | |

2 Save the method to a new method name, *iii*Sulfas_TriggerOpt.m, where *iii* are your initials.

~

- a Click the Method > Save Method command.
- **b** Type iiiSulfas_TriggerOpt.m.
- c Click the Save button.

Task 3. Create a Triggered Dynamic MRM method from an existing database

| Steps | | | etailed Instructions | Comments | | |
|-------|--|-------------|---|----------|---|--|
| 3 | Review the method in the Dynamic MRM Viewer dialog box. | a b c | Right-click the Scan segments table and click Edit DMRM Method . The Dynamic MRM Viewer dialog box is opened. Type 200 for the Cycle time . This value is shown in the Acquisition tab. Click between the Primaries only button and the All transitions button if the Dynamic MRM Statistics information is not updating. Then, click the All transitions button. | • | The compounds in the Optimizer program were overwritten by the compounds that you updated in the Database Browser program. You can modify the Cycle time and see how the Minimum Dwell Time is changed. If the Minimum Dwell Time is less than 5 ms, and especially if it is less than 2 ms, then signal-to-noise is poor. A Dwell Time of 8 ms per transition is fine. | |

| Compound: (All) | | E Compos | | ۵n | | a na s | a | | | | | Dynamic MRM Stat | stics |
|--------------------|-----------------------|--------------|-----------------|-------|--------|-------------|---------------------------|------------------|--------|-------|------|----------------------------|-------------|
| sompound. [(en) | | Compor | ind choop. If | (-)() | - | | 1 | | | | | Total MRMs | 30 |
| C | X C | Precursor | Product | DT. | BT | Prima 🕁 | T.: | Thursday | | er. | C414 | Minimum Concurrent MRMs | 7 |
| Compound Group | v Compound Name v | lon | lon | BI . | Window | ry ¥ | ingger v | Infeshold | Frag | LE | LAV | Maximum Concurrent MRMs | 21 |
| | sulfachloropyridazine | 285.00 | 155.70 | .650 | 1.000 | V | V | 48875 | 380 | 10 | _ | Minimum Dwell Time | 8.61 ms |
| | sulfachloropyridazine | 285.00 | 107.90 | .650 | 1.000 | V | | | 380 | 24 | _ | Maximum Dwell Time | 27.66 ms |
| | sulfachloropyridazine | 285.00 | 129.90 | .650 | 1.000 | 1 | | | 380 | 24 | | Minimum Cycle Time | 124.26 ms |
| | sulfachloropyridazine | 285.00 | 91.90 | .650 | 1.000 | 17 | | | 380 | 24 | | | |
| | sulfachloropyridazine | 285.00 | 79.80 | .650 | 1.000 | | | | 380 | 48 | | | |
| | sulfachloropyridazine | 285.00 | 64.80 | .650 | 1.000 | | | | 380 | 48 | _ | | |
| | sulfadimethoxine | 311.00 | 155.70 | 2.030 | 1.000 | V | V | 169666 | 380 | 15 | | Parameters | |
| | sulfadimethoxine | 311.00 | 107.90 | 2.030 | 1.000 | V | | | 380 | 20 | _ | Cycle time 200 | ms |
| | sulfadimethoxine | 311.00 | 244.80 | 2.030 | 1.000 | | | | 380 | 12 | | Calculations include: | |
| | sulfadimethoxine | 311.00 | 229.70 | 2.030 | 1.000 | | | | 380 | 20 | | C Primaries only | transitions |
| | sulfadimethoxine | 311.00 | 217.70 | 2.030 | 1.000 | | | | 380 | 16 | | | |
| | sulfadimethoxine | 311.00 | 172.90 | 2.030 | 1.000 | 12 | | | 380 | 24 | | Review Tools | |
| | sulfadimethoxine | 311.00 | 91.90 | 2.030 | 1.000 | | | | 380 | 32 | | | _ |
| | sulfadimethoxine | 311.00 | 79.90 | 2.030 | 1.000 | | | | 380 | 48 | | C Override RT window 1 | min |
| | sulfadimethoxine | 311.00 | 64.80 | 2.030 | 1.000 | | | | 380 | 48 | | Check minimum data pts 64 | pts |
| | suifamethazine | 279.00 | 185.70 | .980 | 1.000 | 7 | 1 | 183517 | 380 | 11 | | | |
| | sulfamethazine | 279.00 | 123.90 | .980 | 1.000 | V | | | 380 | 24 | | Split Method | |
| | sulfamethazine | 279.00 | 212.80 | .980 | 1.000 | 100 | | | 380 | 20 | | E o n u u | |
| | sulfamethazine | 279.00 | 155.90 | .980 | 1.000 | | | | 380 | 12 | | 1 Split method | |
| | sulfamethazine | 279.00 | 107.90 | .980 | 1.000 | 100 | | | 380 | 24 | | Split by: Minimum Dwell Ti | me 👻 |
| | sulfamethazine | 279.00 | 91.90 | .980 | 1.000 | | | | 380 | 24 | | | |
| | sulfamethazine | 279.00 | 79.80 | .980 | 1.000 | 111 | | i i | 380 | 48 | | Number of methods 2 | |
| | sulfamethazine | 279.00 | 64.90 | .980 | 1.000 | | | | 380 | 48 | | Max concurrent MRMs | 0 |
| | sulfamethizole | 271.00 | 155.80 | .370 | 1.000 | V | V | 68236 | 380 | 6 | | | |
| | sulfamethizole | 271.00 | 92.00 | .370 | 1.000 | V | | | 380 | 28 | | Min dwell time 5 | |
| | sulfamethizole | 271.00 | 177.80 | .370 | 1.000 | | | | 380 | 12 | | Split Method: | • |
| | sulfamethiznle | 271 00 | 115 90 | 370 | 1 000 | 100 | | | 380 | 16 | | | _ |
| | | | | | | | • | | - | | , | | |
| ot type Concurrent | MRMs 💌 | 🔽 Select tra | nsitions on Cli | sk | | | | | | | | | |
| | | | | | Cor | ncurrent MF | IMs vs Retent | on Time | | | | | |
| 20- | | | | | | | | | | | | | |
| 15- | | | | | | | | | | | | | |
| | | | L | | | | | | | | | | |
| 10- | | | | | | | | | | _ | - | | |
| 5- | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| 0.1 | 0.2 0.3 0.4 0.5 | 0.6 0.3 | 0.8 | 0.9 i | 1.1 | 1.2 F | 1.3 1.4 Retention Time | 1.5 1.6 (min) | 1.7 1. | 8 1.9 | 2 | 21 22 23 24 25 | 2.6 2.7 |
| | 1 | 6 | | | | | | | | | | | 1 |
| Add Compounds | Save Split Methods | | | | | | | | | | | Reset Default | Close |

Task 3. Create a Triggered Dynamic MRM method from an existing database

| St | teps | Detailed | nstructions | Comments |
|----|--|--|---|----------|
| 4 | Review the Trigger Thresholds to verify that they are appropriate. | a Do an Trigger b Right-o and cli c In the | injection to make sure that the Thresholds are set properly. Slick the Scan segments table ck Update DMRM Method . MRM Update Options dialog | |
| | | box, se Thres ł | elect True for Update old?. | |
| | | d Entert Height | he value for the Percent of for the Trigger Threshold. | |
| | | e Select acquir | the data file that you just ed. | |
| | | f Click C | K. | |

| Method Uptions | C-li- |
|--|--------------------------|
| Add new Compound? | False |
| Cuela Tima | 30 |
| Distantian Time | 250 |
| Ladate Retention Time? | Taus |
| Update Retention Time) (Indow? | True |
| Scale Factor of BT Window to Peak Width | 3 |
| Retention Time Window Threshold | 1 |
| Retention Time Window Threshold Unit | Minutes |
| Trigger Threshold | minutes |
| Undate Threshold? | True • |
| Percent of Height | 5 |
| Scale Factor | 1 |
| Trigger Window | |
| Update Trigger Window? | True |
| Trigger Window Options | RetentionTime |
| Absolute value (mins) | 0.5 |
| Percent value | 0 |
| Scale Factor | 1 |
| Update Threshold? Indicates whether to update the trigger MRM | threshold of the method. |

Task 1. Use the Optimizer Software to optimize acquisition parameters

Exercise 4 – Optimize Acquisition parameters

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

Task 1. Use the Optimizer Software to optimize acquisition parameters

The Optimizer Software helps you optimize acquisition parameters. Specifically, it automates the selection of the best precursor ions, the optimization of the fragmentor voltage for each precursor ion, selection of the best product ions, and optimization of collision energy values for each transition for a list of compounds you specify.

To do this task, you first need to create the method *iii*Sulfamix MRM_10.m in "Task 5. Find optimum collision energy for MRM acquisition" on page 30. You do not need to acquire the data file.

The Fragmentor Voltage for the 6490 is set automatically during Autotune. The Fragmentor voltage for a 6490 is not optimized. The Fragmentor parameters and results will not be displayed for a 6490 instrument.

| St | eps | Detailed Instructions | Comments |
|----|--|---|--|
| 1 | Start the MassHunter Optimizer software. | • Double-click the Optimizer icon. 🙀 . | If you are optimizing peptides, use the Optimizer for Peptides program. |

Task 1. Use the Optimizer Software to optimize acquisition parameters

| teps | Detailed | Instruct | ions | | Comments |
|--|--|----------|------------|----------|----------|
| MassHunter Optimizer (OptimizerProject3) | | | | | |
| File Edd View Import/Esport Optimization Tools He | * | 0 | | | |
| Optimizer Setue Precursor los Selection Product los Selection Com | pound Setup | | | | |
| Sample introduction Prection (with or without column) | Path for data files C. MassHunter data Optimizer | | | | |
| Advantic inflution using loss intention | Acq Method | Polarity | Ion Source | Comments | |
| | C'MassHunter/Methods/default.m | Positive | ESI | | |
| Fire Step (-/-5 steps anual cases) Fand Collean Trage Collean Trage Collean Trage Converts exiting Start han predicted value Converts exiting Formula ED = $\frac{m^2}{100}$ # + + | | | | | |
| Cell Accelerator Votage | | | | | |
| | | | | | |

Task 1. Use the Optimizer Software to optimize acquisition parameters

| Steps | Detailed Instructions | Comments |
|------------------------------------|--|---|
| 2 Set the optimization parameters. | a Click the Optimizer Setup tab. b Set the Sample introduction method to Injection. c Set the Fragmentor ramp parameters as follows: Set the range for ramping the Fragmentor values from 90 to 135. Clear the Fragmentor Fine check box. d Set the range for ramping the Collision Energy from 0 to 40 V. e Select a Path for data files to store the optimization run data. f Right-click the table on the right and select Add Method from the shortcut menu. g Click the button on the right side of the Acq Method cell to open the Open Method dialog box. | Fine optimization refines the coarse ramping values and provides better optimization but takes longer to run. The data can be displayed later with Agilent MassHunter Qualitative Analysis software. The Fragmentor Voltage is not optimized for an Agilent 6490 Triple Quadrupole. It is set automatically when you Autotune. The Fragmentor parameters and results for a 6490 are not shown in the Optimizer program. |
| | h Select the method created in the previous exercise <i>iii</i>Sulfamix MRM_10.m and click OK. The Polarity and Ion Source will be filled in from the values set in the selected method. i Check to make sure that the Ion | |
| | Source from the method matches the physical configuration of your instrument. | |
| | j Repeat step f to step i to select additional methods. | |

Task 1. Use the Optimizer Software to optimize acquisition parameters

Steps

3 Select the precursor ions

Detailed Instructions

- a Click the **Precursor Ion Selection** tab.
- **b** Select the **Positive ions** +H adduct.
- c Select the Charge state of 1.
- **d** Set the search priority of the precursor ions.
- e (optional) To exclude certain masses from consideration, click Exclude masses at the bottom of the screen. Enter the m/z Values to exclude separated by commas and/or enter a Minimum abundance value in counts.

Comments

- Mark the Use most abundant precursor ion check box to use the most abundant precursor ion.
- Clear the Use most abundant precursor ion check box and use the Up and Down arrow buttons to set the search order (ions at the top of the list are given more priority).
- You can also enter Neutral Losses to exclude (for example H₂0).

| etive ions (with priorities) | Negative ions (with priorities) | Charge state | 📝 Use most abundant precursor ion | |
|-------------------------------|---------------------------------|-----------------------|-----------------------------------|--|
| Do not exclude masses | | | | |
| Exclude masses ecumor ion | | | | |
| m/z value(s) Mnmum abundance | counta | (separated by common) | | |
| | | | | |
| | | | | |
| | | | | |

Task 1. Use the Optimizer Software to optimize acquisition parameters

| St | eps | D | etailed Instructions | Comments |
|----|-------------------------|-------------|--|----------|
| 4 | Select the product ions | a b c | Click the Product Ion Selection tab. Enter a Low mass cut-off value. Select Mass (m/z) of 60 m/z. To exclude certain masses from consideration, click Exclude masses option at the bottom of the screen. Enter the m/z Values to exclude separated by commas and/or enter a Minimum abundance value in counts. If desired, you can also enter Neutral Losses to exclude, for example H ₂ 0. | Comments |
| | | | Enter a formula in the box and click the button to add it to the list. | |

| | 🐔 🕍 🐔 🕈 | is % 🐐 🔝 | M 🚮 🔇 | | |
|---|------------------------------------|----------|-----------------------|------------------|--|
| nizer Setup Precursor Ion Sele | tion Product Ion Selection Compour | nd Setup | | | |
| [Mass (m/z)] The Curson mass (m/z) | © | | | | |
| Do not exclude masses Exclude masses | | | | | |
| educt ion | | | (separated by commas) | Neutral Losses | |
| I Minimum abundance | counts | | | [2] H2O | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

Task 1. Use the Optimizer Software to optimize acquisition parameters

Steps

- 5 Set up a compound list. The formula for the four Sulfa Drugs are:
- Sulfamethizole C₉H₁₀O₂N₄S₂
- Sulfamethazine C₁₂H₁₄O₂N₄S
- Sulfachloropyridazine C₁₀H₉O₂N₄SCI
- Sulfadimethoxine C₁₂H₁₄O₄N₄S

Detailed Instructions

- a Click the Compound Setup tab.
- **b** Clear the **Show results summary** check box above the table while you set up the compound list.
- c Right-click the table and select Add Compound from the shortcut menu to add a row to the end of the table.
- d Enter Sulfamethizole as the Compound Name.
- e Enter Sulfa drugs as the group name in the Groups column.
- f Enter C9H1002N4S2 as the Formula of the compound. The mass is calculated.
- g Enter the Sample Position for the new compound.
- h (optional) Enter an Optimization dwell time value to set longer or shorter cycle times.
- i Repeat the steps above to add the other three sulfa drugs to the table.
- j Mark the **Select** columns for the compounds (rows) to use for optimization.
- k Save the compound list to the database or to the current project.

Comments

- Compounds are global to all projects. Compound information such as name, group, formula, and mass in one project will be reflected in the entire database.
- If no methods or ions are specified here, then optimization for the compound uses the methods from the Optimizer Setup tab and information from the Precursor Ion Selection and Product Ion Selection tabs to generate the ions.
- You can also enter the monoisotopic mass in the Mass column instead of the Formula.

Task 1. Use the Optimizer Software to optimize acquisition parameters

| Steps | Detailed Instructions | Comments |
|--|--|---|
| MassHunter Optimizer (Optimizer/Project)) File Edit View Import/Export Optimization Tools H Tools I and the Amport/Export Optimization Tools H Contract of the Amport/Export Optimizer (Optimizer) Optimizer State Precurso Ion Selection Product Ion Selection Cont | np 🐅 🌸 🍕 🚮 🕞 🖓 | |
| Show result summay | Formula Mass Sample Position 1007H42 27000 P1-43 1002H42 27000 P1-43 1002H42 38401 P1-43 11402H43 31007 P1-41 11402H43 31007 P1 | 10t Experted peak width (pase) Error na Frag free cycle time Error ma Product scon cycle time ma Product tion Cycle time ma Product tion Cycle time ma Product tion Cycle time ma |
| Project Name : OptimizerProject 1 Optimizer 1 | Ready Current Record | All Records |
| Start the optimization process. | Click the Start Optimization button () on the toolbar or Click the lon Breakdown Profile b () on the toolbar. | n • |
| Review results. | a Click the Compound Setup tab. b Mark the Show results summary check box above the table. c Review the following values for e transition ion in the Compound Ta Fragmentor Collision Energy d Review the printed optimization r | (optional) Use the Agilent MassHunter Workstation Qualitative Analysis program to look at the data. See the online Help for the Optimizer program or the Optimizer Quick Start Guide to learn how to import optimization results to acquisition for MRM time segments. |

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

The "Source and iFunnel Optimizer" Software helps you optimize acquisition parameters for the source and iFunnel.

To do this task, you first need to create the method *iii*Sulfamix_dMRM2.m in "Task 4. Create a Dynamic MRM method from an MRM method" on page 39. You do not need to acquire the data file. When you use this software, a worklist for each of the parameters being optimized is added to the Study Manager program.

| S | teps | D | etailed Instructions | C | omments |
|---|--|------------------|---|---|--|
| 1 | Start the MassHunter Data Acquisition software and load the iiiSulfamix_dMRM2.m method. Save this method to a new name. | a b c d | Start the Data Acquisition program. Make sure that Acquisition appears as the selection in the Context text box. If Tune is the selection, click Acquisition from the Context dropdown menu in the Combo bar . Load the <i>iii</i> Sulfamix_dMRM2.m method. Save this method to the name <i>iii</i> Sulfamix_SourceOpt.m . | • | The first step is to create a template method. This method is used when you are optimizing the source and iFunnel parameters. |
| 2 | Edit the Source parameters | с 9 | Click the 000 tob | | |
| 2 | Eult the Source parameters. | a h | Click the Source tab on the OOO tab | | |
| | | | Medify the noremotors to the | | |
| | | C | recommended starting parameters for source optimization. These parameters are shown in the following image. | | |

| Properties DA Sampler Sampler Proteatment Binary Pump Colu | mn Comp. DAD QQQ |
|---|--|
| Tune Ble Stop time Marres Tune with Summers tune with Stop time Min Muka Pump Crime Min Maka Pump Crime Stop time To table Crime Stop time AS 551 Image: Stop time In totato Image: Stop time In totato Image: Stop time If and the stop time Image: Stop time If and time Image: Stop time </td <td>Acquisition Severe Oronatogram Instrument Diagnostics Source parameters Gas Temp: 150 C 150 C Gas Temp: 151 C 150 C High Pressure RF Fostive Negative Metudien: 200 pri 2000 pri 2000 pri Low Pressure RF Footive Negative Sheath Gas Temp: 150 C 350 C Sheath Gas Temp: 100 V Low Pressure RF Footive Negative Copy Paste Paste Date to All Segments Paste Paste<</td> | Acquisition Severe Oronatogram Instrument Diagnostics Source parameters Gas Temp: 150 C 150 C Gas Temp: 151 C 150 C High Pressure RF Fostive Negative Metudien: 200 pri 2000 pri 2000 pri Low Pressure RF Footive Negative Sheath Gas Temp: 150 C 350 C Sheath Gas Temp: 100 V Low Pressure RF Footive Negative Copy Paste Paste Date to All Segments Paste Paste< |

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

Steps **Detailed Instructions** Comments

- 3 Create template method.
- a Click the Acquisition tab.
- **b** Select a single ion for each compound for the optimization.
- c Save the method.

· A transition for each compound is already included in the Dynamic MRM method.

| Proparties DA Sampler Sampler Pretreatment Pinau Prop Colu | mn Como DAD DE | 10 | | | | | | | | | | | |
|--|--------------------------------------|--------------------------------|---------|------------------|-----------|-----------------|-------------|-------------------|-------------------|-------------|---------------------|-----------------------------|----------|
| Tune Re | Acquisition Source | Chromatogram Instru | ment | Diagnostics | | | | | | | | | |
| atunes tune xml | - Scan segments | on on degran nore | inora | Diagnoonee | | | | | | | | | |
| F Line Dolu Browse 66 C 1 min | Compound Group | Compound Name / | ISTD? | Precursor Ion 🗸 | MS1 Res | Production V | MS2 Res | Ret Time (min) | Delta Ret Time | Fragmentor | Collision Energy | Cell Accelerator Voltage | Polarity |
| Ion source | • | sulfachloropyridazine | | 285 | Unit | 155.7 | Unit | 0.65 | 1 | 380 | 10 | 7 | Positive |
| | | sulfadimethoxine | | 311 | Unit | 155.7 | Unit | 2.03 | 1 | 380 | 15 | 7 | Positive |
| AUS ESI | | sulfamethazine | | 279 | Unit | 185.7 | Unit | 0.98 | 1 | 380 | 15 | 7 | Positive |
| Time segments | | sulfamethizole | | 271 | Unit | 155.8 | Unit | 0.37 | 1 | 380 | 10 | 7 | Positive |
| Ime Crwv (*) Ewv (*) 1 0 Dynamic MRM To MS 200 0 | | 6 | | | | | | | | | | | |
| cycles/s ms/cycle | Dynamic MRM Parame Cycle Time 200 | ters Total MRMs = 4 M ms | 4ax Con | current MRMs = 3 | 8 Min/Max | Dwell = 65.78 m | s/199.20 ms | | | Triggered M | IRM red | Repeats 3 | |

4 Start the MassHunter "Source and • Double-click the Source Optimizer icon iFunnel Optimizer" program.



| roject pr | arameters | | | | | | | |
|-----------|-------------|-------------------------|------------------------|--------------|-----------------------|----------------|----------|-----------|
| | oficiae co | ethod CM | and the start mathe | de defe it o | | | _ | 0-munt |
| | | 0.00 | and the lost strain in | | | | _ | browse |
| P | roject Fold | der C:\M | assHunter\data | | | | | Browse |
| P | roject Na | me MyTe | et. | | | opend timest | атр | |
| | | | | | | | | |
| strumen | t paramet | ers | | | | | | |
| | V | Types | PreWat(nin) | Replicate | StepWat (min) | StartValue | EndValue | Step Size |
| ۰. | | High Pressure RF | 0 | 1 | 0 | 70 | 210 | 20 |
| | V | Low Pressure RF | 0 | 1 | 0 | 40 | 160 | 20 |
| | V | Gas Temp | 30 | 1 | 20 | 120 | 230 | 30 |
| | V | Gas Row | 30 | 1 | 0 | 11 | 20 | 2 |
| | V | Nebulizer | 0 | 1 | 0 | 20 | 40 | 5 |
| | J | Capillary | 0 | 1 | 0 | 1500 | 4500 | 500 |
| /orklet p | varameters | a My Test Samole Nan | 0 | | | Sample | Postion | Val 1 |
| | | | | Worklat | position of data file | e used for cal | Ibration | 1 |

When this program starts, it automatically selects the default.m method. This method is not set up for an Agilent Jet Stream source, so no Agilent Jet Stream parameters are shown in the Instrument parameters table.

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

| Steps | Detailed Instructions | Comments |
|---|---|----------|
| 5 Select the template method, <i>iii</i> Sulfamix_SourceOpt.m. | a Click the Browse button. The Brow For Folder dialog box is opened. b Select the <i>iii</i>Sulfamix_SourceOpt method. Click the OK button. c If the ion source in the method is different than the ion source in the last metric automatical back a ware | m |

message is opened. Click **OK**.

| | Tool | Help | | | | | | | |
|----|---------|-------------|------------------|---------------|-------------|-----------------------|----------------|----------|----------|
| | 1 | | | | | | | | _ |
| 'n | oject p | arameters | | | | | | | |
| | (| Optimize m | ethod D:\Ma | ssHunter'Meth | sds\FamGuid | le\S_0pt_6490_4 | JS.m | | Browse |
| | 1 | Project Fol | der D:'VMa | ssHunter\data | | | | | Browse |
| | , | Project Na | MyTe: | t | | V A | ppend timest | amp | |
| ne | strume | nt paramet | ers | | | | | | |
| | | V | Types | PreWait(min) | Replicate | StepWait (min) | StartValue | End/alue | StepSize |
| | ۲. | | Gas Temp | 0 | 1 | 0 | 130 | 240 | 30 |
| | | V | Gas Flow | 0 | 1 | 0 | 11 | 20 | 2 |
| | | V | Nebulizer | 0 | 1 | 0 | 15 | 50 | 5 |
| | | V | Capillary | 0 | 1 | 0 | 1500 | 4000 | 500 |
| | | 4 | Cah Pressure RF | 0 | 1 | 0 | 70 | 210 | 20 |
| | | V | Low Pressure RF | 0 | 1 | 0 | 40 | 160 | 20 |
| | | V | Sheath Gas Temp | 0 | 1 | 0 | 200 | 400 | 50 |
| | | V | Sheath Gas Flow | 0 | 1 | 0 | 6 | 12 | 2 |
| | | V | Nozzle Voltage | 0 | 1 | 0 | 0 | 2000 | 500 |
| | | | | | | | | | |
| | (wkEnt | naramatar | | | | | | | |
| | Sampl | e Name | MyTestSampleName | | | | Sample F | Position | Vial 1 |
| | | | | | Worklist | position of data file | e used for cal | ibration | 1 |
| | | | | | | | | | |
| | | in sin Mail | webs - | | Submit | | | | |

The Sheath Gas Temp, Sheath Gas Flow, and Nozzle Voltage are all specific to the Agilent Jet Stream. If you do not have an Agilent Jet Stream source, these rows are not included in the table.

The High Pressure RF and Low Pressure RF are only included if the QQQ model is a 6490.

- 6 Change the order of the rows in the Instrument parameters table to the following:
- · Ion Funnel parameters
- Sheath Gas temperature and flow
- · Gas temperature and flow
- Nebulizer
- Capillary
- Nozzle Voltage

- **a** If necessary, select the row that shows the **High Pressure RF** parameter.
- **b** Drag this row to the top of the table. Both of the lon Funnel parameters are moved together.
- c Verify that the order of the rows in your table is as indicated.
- The order of the parameters in the Instrument parameters table is the order that the parameters are optimized. You want to optimize the parameters that have the greatest effect on the source optimization first. The Ion Funnel parameters have the greatest effect, so you move those parameters to the top of the list.
- By default, the parameters are in the optimized list.

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

Steps

Detailed Instructions

Instrument parameters V PreWait(min) Replicate StepWait (min) StartValue EndValue StepSize Types Low Pressure RF 160 20 V 0 40 1 400 50 Sheath Gas Temp 30 20 200 Sheath Gas Flow 30 0 10 12 1 V 30 20 120 230 30 Gas Temp V 20 Gas Flow 30 0 11 2 V 20 40 5 Nebulizer 0 0 500 1 Capillary 1500 4500 0 0 V 2000 500 Nozzle Voltage 0 0

The High Pressure RF and the Low Pressure RF are always optimized together. If you move one of these rows in the table, the other row is also moved. All possible combinations of the High Pressure RF and Low Pressure RF are tried to find the optimal values. For this example, the High Pressure RF parameter has 8 different parameter settings (70, 90, 110, etc.). The Low Pressure RF parameter has 7 different parameter settings. So, the program automatically creates 56 (8 * 7) different methods (1 for each parameters.

Comments

For each row in the table, verify:

- PreWait (in minutes).
- Replicate.
- StepWait (in minutes).
- StartValue.
- EndValue.
- StepSize.

- When the study for each parameter is loaded for the first time but before you run the first run, you wait the **PreWait** number of minutes before starting the run. Some parameters (that are electronic) stabilize almost instantly (in milliseconds), so you do not need to wait. For flows and temperatures, you want to have a **PreWait** before you run the study.
- You also want to wait for temperature parameters in between changing the parameter to a different value, so you also set the StepWait (in minutes).

8 Save the Instrument parameters.

7 Review the values for each

parameters table.

parameter in the **Instrument**

- **9** Modify the Instrument parameters table to only modify one parameter for this task. This task only optimizes the **Capillary** voltage.
- a Click File > Save As (*.opt).
- **b** Enter the name for this set of instrument parameters.
- c Click OK.
- a Mark the check box next to the **Capillary**.
- **b** Clear the check boxes next to all of the other parameters.

 For this example, you optimize the Capillary. Usually, you optimize the parameters in the order specified in the Instrument parameters table.

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

| eps | | | | | De | etailed | Instr | uctions | Comments |
|------------------|----------------------|----------------|--------------------|-----------|-----------------|--|--|--|---|
| ument par | rameters | | | | | | | | |
| | T T | nes | PreWat/min) | Replicate | Sten Wait (min) | StartValue | FodValue | Sten Size | |
| | High Pre | ssure RF | 0 | 1 | 0 | 70 | 210 | 20 | |
| | Low Pre | ssure RF | 0 | 1 | 0 | 40 | 160 | 20 | |
| | Sheath (| àas Temp | 30 | 1 | 20 | 200 | 400 | 50 | |
| | Sheath | Gas Flow | 30 | 1 | 0 | 10 | 12 | 1 | |
| | Gas | Temp | 30 | 1 | 20 | 120 | 230 | 30 | |
| | Gas Not | How | 30 | 1 | 0 | 11 | 20 | 2 | |
| | Car | illary | 0 | 1 | 0 | 1500 | 40 | 500 | |
| | Nozzle | Voltage | 0 | 1 | 0 | 0 | 2000 | 500 | |
| Set t Proje | he Proj ect Nan | ect Fa 1e. | older an | d the | a b c | Selec exam Enter (optio times | t the I ple, se a Pro nal) N tamp | Project elect \ <i>N</i> ject Na /lark the check b | If you mark the Append timestamp check box, then a time stamp is automatically added to the Project Name when you click the Submit button. |
| Projec Projec | ct Folder ct Name | C:\Ma MyTes | ssHunter\data t | | | Append times | amp | Browse | |
| Set t | he Wor | klist p | parame | ters. | a b c | Type 1 Type 1 Type 1 used | he Sa he Sa he W for ca | imple N imple P orklist _I libratio | If you mark the Append timestamp check box, then a time stamp is automatically added to the Project Name when you click Submit. For each parameter that is optimized, a batch file is created fo Quantitative Analysis. One of the injections is considered 100% of the starting value. The value of Worklist position of data file used for a solitoria attraction batch data |
| | | | | | | | | | file to use. If you enter 1, then the |
| | | | | | | | | | file to use. If you enter 1, then the data file from the first row is used. |
| list parame | ders | | | | | | | | file to use. If you enter 1, then the data file from the first row is used. |

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

| Steps | Detailed Instructions | Comments |
|--|--|--|
| 12 Create the methods and submit the study to the Study Manager. | a Click Create Methods. b Click Submit. | When you click Create Methods, a message at the bottom of the main window states how many Methods were created, how many Injections are involved, and the Estimated time. The Estimated time is only an approximation. |
| Project created: D:\MassHunter\data\S_Opt_vcp_2012912_1 6 metho | ds 6 injections Estimated time: 21 minutes | |

The estimated time includes the Stoptime for the method plus one minute per injection. It does not consider the Posttime specified in the method. Also, it does not include the PreWait nor the StepWait that you entered in the Instrument parameters table.

- **13** Review the study (or studies) submitted to Study Manager.
- **a** Open the Study Manager program.
- **b** Select a row in the Pending Studies table.
- c Right-click the row and click Edit Worklist From Study.
- d Review the worklist in the Edit Worklist dialog box. Click **Save**.
- A study is submitted for each parameter that you marked in the Instrument parameters table.
- Only one study is created for the High Pressure RF and Low Pressure RF parameters.

| Home | Settings | N | AassHunter Study Ma | nager | | | - = X |
|--|--|----------------------------|-------------------------|----------------------------|-----------------|---------------|------------|
| Start Stop | Stopped Status | <u>&</u> Submit | Logbook Lock Conso | Help Ie | | | |
| Stu | idy Execution | Study Submission | Study Actions | Help | | | |
| Name: | | Path | | | Eel | Timo Romainii | n (min) |
| Submitter: | | Plate Assi | ignment: | | 200 | | ig (iiiii) |
| | | | | | | 0.0 | |
| | | | | | | | |
| Status: | | | | | | | |
| Status: | | | | | | | ¥ |
| Status: | Completed Studies | | | | | | 3 |
| Status: Vending Studies Name | Completed Studies Path | Submitter Pile | oke Assignment Es | I. Study Duration | Est. Start Time | Sample Count | ¥ |
| Pending Studies Nome Copil.logy 1 | Completed Studies Path Dr.MassHunteNd | Submitter Ple admin 31 | ole Assignment Es 00 | t. Study Duration 21.00 | Est. Start Time | Sample Count | 8 |
| Vending Studies Name Discussions | Completed Studies Path D'Messihurdenid | Submitter Ple action 31 | Ve Assignment Es 00 | I. Study Duration | Est. Start Time | Sample Count | * |

The name of the study is the Instrument parameter that is being optimized. A separate study is added for each parameter that is being optimized.

You can examine or edit the worklist for the study. You right-click the line in the Pending Studies table and click Edit Worklist from Study.

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

| Step | S | | | Deta | ailed Instructions | | | Comm | ients |
|---------|--------|------------------|-----------------|-----------------------|---------------------------|--------------|------------|------------|----------------------------------|
| Edit Wo | orklis | t Sampla Namo | Sample Desition | Method | Dete Edo | Sample Turne | Lavel Name | Commont of | |
| 1 | | sulles | Viel 21 | S Ord Capillan/2000 m | S Oct Capillas (2000, 1 d | Celibration | Leverriane | comment a | The covint that is you at the on |
| 2 | 2 | sulfac | Vial 31 | S Opt Capillar/2500 m | S Opt Capillar/2000_1.d | Sample | | | The script mat is run at the en |
| 3 | v | sulfas | Vial 31 | S Opt Capillar/3000 m | S Opt Capillary2000_1.d | Sample | | | of the worklight erected the |
| 4 | v | sulfas | Vial 31 | S Opt Capillary3500 m | S Opt Capillary3500 1.d | Sample | | | of the worklist creates the |
| 5 | v | sulfas | Vial 31 | S Opt Capillary4000 m | S Opt Capillary4000 1.d | Sample | | | Quantitativa Analysia hatah |
| 6 | V | sulfas | Vial 31 | S Opt Capillary4500.m | S Opt Capillary4500 1.d | Sample | | | Quantitative Analysis batch |
| | | | | | D ₂ | | | | |
| | _ | | | V Same | Vorklist | | | | |

- **14** Modify the Study Manager parameters to run a standby script when the study completes and then start the Study Manager.
- **a** Click the **Settings** tab in the Ribbon.
- **b** Mark the **Enable standby script execution on idle** check box.
- c Click the "..." button to select the script to execute.
- d Select SCP_InstrumentStandby and click the OK button.
- e Enter 1 for the Wait for time.
- f Click the Start button if necessary.
- When the Study Manager is not running a study for the time specified, then the script you select is executed.

| ped Subi Study Sul | mit Logbook amission Study Ac Patr: Plate Assignment | Lock Help Console Help | Est. T | īme Remainin | a (min) |
|--------------------------|--|---|---|---|--|
| ped Sub Study Sul | mit Logbook amission Study Ac Patr Plate Assignment | Lock Help Console Help | Est. T | īme Remainin | a (min) |
| study Sub | mit Logbook omission Study Ac Path: Plate Assignment: | Lock Help Console * tions Help | Est. T | ime Remainin | a (min) |
| Study Sul | Path: Plate Assignment: | tions Help | Est. T | ime Remainin | a (min) |
| | Path: Plate Assignment: | | Est. T | Time Remainin | a (min) |
| | Path: Plate Assignment: | | Est. T | Time Remainin | a (min) |
| | Plate Assignment: | | Est. 1 | Time Remainin | a (min) |
| | Plate Assignment: | | | | 3 () |
| | | | | 0.0 | |
| | | | _ | 0.0 | |
| | | | | | |
| | | | | | 8 |
| | | | | | |
| | | | | | |
| udies | | | | | |
| Submitter | Plate Assignment | Est. Study Duration | Est. Start Time | Sample Count | |
| ssHunter\d., admin | 31 | 00.21.00 | | 7 | |
| | tudies Submitter sstHunter\d. admin | tudet Subratter Plate Assignment raktart mild.n. admin 31 | tudet Submitter Plate Assignment Eut. Study Duration miktant mid.1, admin 31 00/21:00 | tudet Submiter Plote Assignment Ext Study Duration Ext Stud Time Inskantendal Johann 31 (2021-00) | Notes Submitter Plate Assignment Est Study Duration Est Start Time Sample Count Instructionscien 31 002100 2 2 |

The name of the study is the Instrument parameter that is being optimized. A separate study is added for each parameter that is being optimized.

You can examine or edit the worklist for the study. You right-click the line in the Pending Studies table and click Edit Worklist from Study.

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

Steps

Detailed Instructions

- 15 When the study completes, stop the Study Manager queue and exit from the Study Manager program.
- **16** Open the data in the Quantitative Analysis program.
- a Click the **Stop > Immediately** command.
- **b** Close the Study Manager program.
- **a** Start the Quantitative Analysis program.
- **b** Click **File > Open Batch**.
- **c** Navigate to the location of the study.
- d Select the Batch file named
- Capillary.batch.bin and click Open.



You specified the Project Folder and the Project Name in the "Source and iFunnel Optimizer" program before you submitted the study.

Comments

The batch file is created automatically at the end of the study.

The "system" folder contains all of the methods that were used in this study.

- Refer to the online Help for the Quantitative Analysis program to learn how to do these tasks.
- In this case, all four compounds optimize at the same setting. Often, different compounds have different optimal settings, and you have to compromise.

17 Review the Batch Table.

- a Switch to Multiple Compound View.
- **b** Add the **Area** column to the table.
- c For each compound, right-click the Final Conc. column and click Plot this column.
- d Examine the Area column and the Final Conc. graph to determine the best capillary voltage.
- e Close the Quantitative Analysis program.
Exercise 4 – Optimize Acquisition parameters

Task 2. Use the "Source and iFunnel Optimizer" program to optimize acquisition parameters

| Steps Deta | | | | | | iled Instructions | | | | | | (| Comments | | | | | | | | | | |
|---|------|--------|-------------------------|--------|-------|-------------------|-------------------------------------|------------------------|----------|---------|-------|-------------------------------|----------|---------------|-------|------------------------|----------|---------|-------|--------------------------|----------|---------|--|
| Batch T | able | | - | | | | | | -0 | | | | | | | | | | | | | | |
| Sample: 🛐 sulfas 🔹 🔹 Sample Type: <all></all> | | | | | | | 👻 Compound: 💷 sulfamethazine 👻 ា IS | | | | | | | "D: 🔤 🙀 🌠 🙀 🔽 | | | | | | | | | |
| Sample | | | | | | | - | sulfamethizole Results | | | | sulfachloropyridazine Results | | | | sulfamethazine Results | | | | sulfadimethoxine Results | | | |
| 1 | 17 | Name | Data File | Туре | Level | Acq. Date-Time | RT | Final Conc. | Accuracy | Area | RT | Final Conc. | Accuracy | Area | RT | Final Conc. | Accuracy | Area | RT | Final Conc. | Accuracy | Area | |
| • | | sulfas | S_Opt_Capillary2000_1.d | Cal | 1 | 9/12/2012 1:36 PM | 0.365 | 100.0000 | 100.0 | 2355970 | 0.649 | 100.0000 | 100.0 | 1745306 | 0.977 | 100.0000 | 100.0 | 6145561 | 2.025 | 100.0000 | 100.0 | 3689776 | |
| same. | ¥ | sulfas | S_Opt_Capillary2500_1.d | Sample | | 9/12/2012 1:42 PM | 0.365 | 82.8676 | | 1952336 | 0.646 | 85.3296 | | 1489263 | 0.970 | 73.8517 | | 4538599 | 2.021 | 99.3833 | | 3667020 | |
| Same. | * | sulfas | S_Opt_Capillary3000_1.d | Sample | | 9/12/2012 1:48 PM | 0.365 | 81.0429 | | 1909346 | 0.646 | 82.1921 | | 1434503 | 0.977 | 69.8619 | | 4293407 | 2.025 | 97.4503 | | 3595698 | |
| Series. | Y | sulfas | S_Opt_Capillary3500_1.d | Sample | | 9/12/2012 1:54 PM | 0.365 | 73.6758 | | 1735779 | 0.649 | 73.5986 | | 1284520 | 0.977 | 62.5807 | | 3845934 | 2.025 | 88.9432 | | 3281804 | |
| Same. | * | sulfas | S_Opt_Capillary4000_1.d | Sample | | 9/12/2012 2:00 PM | 0.365 | 72.4241 | | 1706291 | 0.646 | 71.1248 | | 1241346 | 0.974 | 61.3445 | | 3769965 | 2.025 | 87.5352 | | 3229852 | |
| Carrier . | * | sulfas | S_Opt_Capillary4500_1.d | Sample | | 9/12/2012 2:07 PM | 0.365 | 69.3667 | | 1634258 | 0.649 | 67.9968 | | 1186752 | 0.977 | 58.4264 | | 3590633 | 2.025 | 83.3787 | | 3076487 | |
| | | | | | | | | | | | | | | | | | | | | | | | |

18 (optional) Review the data files in the Qualitative Analysis program.

- De
- a Start the Qualitative Analysis program.
- **b** Open all of the data files in the study.
- c Click Find > Find Compounds by MRM.
- d Select all of the data files and click the **Find** button.
- e Click the Edit > Auto-Color Mode > Single Color per Data File menu item.
- f Clear the check boxes next to the **TIC** for each data file.
- **g** Examine the results in the Chromatogram Results window.
- h Close the Qualitative Analysis program.

- By default, the program selects different colors for different transitions.
- It is clear that the conditions used for the blue chromatograms are the best, and the blue chromatograms are for the data file with the capillary voltage set to 2000.



www.agilent.com

In This Book

This exercise helps you use the Agilent 6400 Series Triple Quadrupole LC/MS system. In this guide, you acquire data and then analyze the results using the Qualitative Analysis program to learn how to develop an acquisition method.

© Agilent Technologies, Inc. 2012

Printed in USA Revision A, November 2012



G3335-90136

