

Introduction to LC-MS/MS technique

Waters HK Office 19-March-2025

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- Chapter Eight: Xevo TQ-S micro Maintenance



Chapter One

TQ-S Micro MS Instrumentation Theory of MS detection

Instrumentation



- Xevo® TQ-S micro Instrumentation
 - Ion Source
 - Interface to vacuum
 - Mass Analyzer
 - Detector
- Atmospheric Pressure Ionization
 - Electrospray Ionization Theory
 - Atmospheric Pressure Chemical Ionization
- Quadrupole Theory

Mass Spectrometry Systems – Xevo TQ-S Micro



Tandem Quadrupole Instruments



Instrument Schematic – Overview





Atmospheric Pressure Ionization (API)



- Electrospray (ESI)
 - Liquid is sprayed from a conductive capillary, to which a high voltage is applied.
 - A spray of charged droplets forms.
 - Droplets evaporate and split until ion evaporation produces gas phase ions
- Atmospheric Pressure Chemical Ionization (APCI)
 - Liquid is passed through a heated tube to be evaporated, producing gas phase molecules.
 - Applying high voltage to a corona pin produces a cloud of ionized nitrogen atoms that ionize the solvent
 - The solvent ionizes the molecules by charge transfer.







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Reduction

Electrospray Ionization Formation of charged droplets



Positive Ion Electrospray – Electrochemical Processes

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- Electrospray droplets carry positive charges away from the capillary tube.
- To balance this flow of positive charges, electrons flow out of the capillary tube.
- An electrochemical oxidation reaction produces electrons from negative ions that are close to the surface of the capillary wall.
- Electrospray can be thought of as an electrochemical process.

Electrospray Ionization Solvent evaporation and droplet fission



Electrospray - Solutions

- Solutions should have a high percentage of organic solvents, such as acetonitrile or methanol.
- Solutions must have some aqueous content and contain ionic species such as hydronium or hydroxyl.

Formation of Gas Phase Ions



- Models for formation of gas phase ions from droplets.
 - Ion evaporation
 - Through evaporation and fission, droplets reduce in size.
 - lons then evaporate from the surface of the droplet.
 - Molecules that are more surface active, more readily form ions in electrospray.

- Charged residue

- o Droplets continue to lose solvent molecules through evaporation until a charged residue remains.
- $_{\circ}$ For an analyte of the form MX, the charged residue is of the form:
- o (M+) n(MX)m



Electrospray Probe Tip







Desolvation Gas Flow

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Detailed ESI Probe



Optimizing Gas Flow Dynamics





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Xevo Z-Spray

- Standard configuration across all Xevo MS detector
- Orthogonal API source for efficient removal of neutrals and maximum sampling of ions



Electrospray and lons in Solution



- A solution process
 - Molecules that have a greater tendency to ionize in solution have stronger electrospray signals.
 - o Certain additives to mobile phases in LC/MS analyses can enhance electrospray signals.
 - For example:
 - Adding formic acid to the mobile phase in positive electrospray LC/MS analyses.
 - This often results in a stronger electrospray signal by aiding in the protonation of analytes in solution.

Electrospray Ionization - Ions Generated in Solution



Acid: HCOOH, low pH buffer

Base + H_3O^+ \leftarrow [Base + H]⁺ + H_2O

Base: High pH buffer, ammonium hydroxide

Acid + HO⁻ \leftarrow [Acid - H]⁻ + H₂O

Choice of Ionization Mode

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Negative ElectroSpray Ions



ESI MS Spectra Example – Positive and Negative Mode



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Multiple Charging in ESI



- Mass spectrometers operate on the basis of the mass-to-charge ratio (m/z).
 - Single charge m/z = (M + H+)/1z
 - Double charge m/z = (M + 2H+)/2z
 - n charge m/z = (M + nH+)/nz
- Isotope peaks of n-charged ions are separated by 1/n m/z.
- Multiple charging extends the effective mass range of the mass spectrometer.

Isotope Resolution for Multiply Charged Species



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Multiply Charged Distributions



Horse Heart Myoglobin



Samples Analyzed in Electrospray Mode

- Typical positive ion samples:
 - Peptides and proteins
 - Small polar molecules (<2000 MW)
 - Drugs and their metabolites
 - Environmental contaminants
 - Dye compounds
 - Various organometallics
 - Small saccharides
- Typical negative ion samples:
 - Various proteins
 - Drug metabolites such as conjugates
 - Oligonucleotides
 - Various saccharides and polysaccharides

Effect of Solution Chemistry on Analyte Response in ESI Waters

- In general, analyte response decreases with an increase in the concentration of certain mobile phase additives. This behavior is referred to as 'ion suppression.'
- Electrolyte ions compete with analyte for charge and occupation on the droplet surface.

Effect of Matrix on Analyte Response in ESI

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Competition between analyte and electrolyte ions for conversion to gas-phase ions decreases analyte response.



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Advantages

- MW confirmation
- High MW determination
- Volatile and non-volatile solutes
- Detection of high MW compounds using multiply charged ions
- Ionic/polar analytes
- Low temperature reduces sample degradation
- Good sensitivity
- Quantitative method
- Suitable for capillary and nano LC flow rates

Disadvantages

- Must form ions in solution
- High salt conditions can suppress ionization
- LC mobile phase additives may affect ionization

ESI

Atmospheric Pressure Chemical Ionization (APCI)

- Low molecular weight (less than 1000 Da)
- Singly-charged species
- Fragmentation, even at low cone voltages
- Mobile phase can be non-polar (normal-phase chromatography)

IonSABRE II[™] APCI Probe Design





APCI Characteristics



- Higher temperature, more aggressive ionization
- Solvent and analyte molecules are in the gas phase
- Ionization takes place in the plasma
- Potentially more sensitive than electrospray with some non-polar molecules
APCI lons

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Positive Ion APCI

- lons similar to those formed in positive ion electrospray are formed as:

```
(M + H)+ or (M + Na)+
```

- Electron abstraction to form M+, free radical cation
- Negative Ion APCI
 - (M H)- ion formed in negative ion electrospray is also produced
 - Free electrons are formed by the corona pin
 - Certain types of molecules can pick up a free electron and become negatively charged without a change in mass. This process is sometimes referred to as M-,free radical anion

APCI and ESI Differences

- Electrospray
 - Ionization in solution
 - Reverse phase or normal phase with post column solvent modifications
 Reverse and normal phase
 - Ionization
 - Probe not heated
 - Capillary voltage
 - Strong mobile phase effect
 - Polar compounds
 - Suitable for thermally labile compounds

- APCI
- Gaseous phase ionization
- - Ionization
 - Heated probe
 - Corona pin
 - Low mobile phase effect
 - Less polar compounds
 - Unsuitable for thermally labile compounds

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ESCi®

- Combines ESI and APCI capability on the Xevo TQ-S micro
- Uses existing ESI probe with the addition of the corona discharge needle
- ESCi provides a choice, through conventional methods, to alternate between ESI-, ESI+, APCI- and APCI+ modes in a single chromatographic run to reduce total analysis time.
- Information on analyte ionization is preserved, for example, when an analyte is only ionized in ESI- mode.



Multiple Scan Functions

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Post Ionization Events

• After ionization, the ions enter the ion block of the Xevo TQ-S micro.



Cone Gas Example - Dextromethorphan



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Traveling Wave Ion Transport





Traveling Wave Ion Transport



Quadrupole Schematic





Quadrupole Assembly





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Quadrupole Example: For three ions passing through the quadrupole, m_1 has a stable trajectory while m_2 and m_3 have unstable trajectories.

Quadrupole Theory

Unit Mass Resolution

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Peak Center = 633.2 Da FWHM = 0.60 Da Mass Resolution is 633.2 / 0.60 = 1055 631 - 632 - 633 - 634 - 635 - 636 - 637m / z

Mass Resolution

Unit Resolution

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Unit Resolution



Unit Resolution

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For most compounds, you do not get a single peak or 2 peaks of equal height.





Chapter Two

Data Acquisition Modes

Data Acquisition Modes

- MS Modes
 - MS Scan
 - SIR
- MS/MS Modes
 - Product Ion Analysis
 - Parent Ion Analysis
 - Multiple Reaction Monitoring
 - Neutral Loss Analysis
 - Survey Scan

🖄 Experiment Setup - c:\m	asslynx\default.pro\acqudb\default.exp	
<u>File Edit View Options</u>	Toolbars Functions <u>H</u> elp	
D 🚅 🖬 🎒 🖉 🗙 🛛		
🗹 SIR 🛛 📝 MRM	📝 MS Scan 🛛 📝 Parents 🛛 📝 Daughters	📝 Neutral Loss 📝 Survey 📝 ScanWave DS
Points Per Peak:		
Total Run Time: 0.00 🔶		
No. Type	Information	
<		

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MS Scan

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MS Scan

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Full Scan Spectra of LC Analysis of a Standard Solution of Thiamethoxam and one of its Metabolites

Spectra taken using 1 second scan time for the range m/z 100 to 400



Spectra of two LC Peaks

Selected Ion Recording (SIR)







SIR Example

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Xevo TQ-S micro MS/MS



- Low energy collisions (simple fragmentation pathways)
- Collision gas of choice is Argon
- Collision gas pressure is normally fixed while the collision energy is used to alter the degree of fragmentation. The collision energy will vary based on the compound
- Sodium and potassium adducts are normally too stable to fragment using low energy collisions.

Product Ion Analysis (Daughter Scan)









Product Ion Analysis





Effect of Changing Collision Energy



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MS/MS of Multiply Charged Ions

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- The most intense ions are normally used for MS/MS even if they are multiply charged
- Multiply charged ions may require higher collision gas pressures than singly charged ions
- Singly-charged fragment ions may have a higher apparent mass (m/z) than their multiply-charged precursor ions



Vancomycin

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MS Spectra of Vancomycin



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MS/MS Spectra of Vancomycin





MS/MS Spectra of Vancomycin



Note: LM & HM values of MS1 were lowered to pass all isotopic forms of the m/z=725 ion into the collision cell. 'Isotope Peaks' are 1 Da apart.


Example of a Doubly Charged Ion

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Example of Product lons from a Doubly Charged Ion



Multiple Reaction Monitoring (MRM)



SIR of Two Compounds

Mixlso_1G14_022 SIR of 1 Channel ES+ TIC 5.95e6 1.31 100 From a Sample that is 60 ng/mL Ketoprofen 60 ng/mL Fenbufen % Ketoprofen Fenbufen 0.80 0. Time 0.90 1.00 1.10 1.20 1.30 MixIso_1G14_023 SIR of 1 Channel ES+ 1.31 TIC 100 6.03e6 From a Sample that is 60 ng/mL Ketoprofen 6 ng/mL Fenbufen %-Ketoprofen Fenbufen ?? Time 1.70 0

0.80

0.90

1.00

1.10

1.20

1.30

1.40

1.50

1.60

Fenbufen



Ketoprofen



Both have a MW of 254

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Comparing MRM and SIR



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Comparing MRM and SIR







Peaks Labeled as Thiamethoxam and Metabolite are not Present in Blank Matrix Samples

Precursor Ion Analysis (Parent Scan)



Precursor Ion Analysis





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For example, this is a MS Scan from a mixture of components.







Neutral Loss Analysis







Tricyclic Antidepressants

Neutral Loss Analysis Example



Neutral Loss Analysis Example



Neutral Loss Analysis Example

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Negative Electrospray Neutral Loss Analysis for loss of m/z = 44 Da from a Mixture of Compounds

Neutral Loss Analysis of Amino Acids by MS/MS

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Neutral Loss Analysis of Amino Acids by MS/MS



Other peaks are from deuterated forms of these amino acids

Survey Scan



- Automatically generates product ion spectra for any components found
- Various triggers can be used
 - MS full scanning
 - Parent ion scanning
 - Neutral loss scanning
- MS and MS/MS data is provided with only one injection, usually on the column.
- MS data is collected until a parent ion of interest elutes.
- MS/MS data for that parent is recorded until the set time or the intensity threshold is reached.
- MS data is collected until another parent ion of interest elutes.

Survey Scan: Screening of Precursor Ion

- Screening of the parent ion fragment can be achieved by:
 - Relative or absolute intensity of a spectrum
 - Specifying included ions or an m/z range
 - Specifying excluded ions or an m/z range
 - Charge state

Survey Scan: Requirements

- m/z range to scan for MS analysis
- m/z range to scan for MS/MS analysis
- Any masses to include or exclude
- Collision energy required for proper fragmentation

Survey Scan Example Chromatogram

SSB02JUN08_011

100-





Survey Scan Example Spectra



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Summary	/	Instrument component			
	Scan type	MS1	Collision cell	MS2	Data type
	MS1 scan	Scan	Inactive	Rf only (acts as ion guide)	Qualitative
	MS scan	Rf only (acts as ion guide)	Inactive	Scan	Qualitative
	SIR	Mass filter	Inactive	Rf only (acts as ion guide)	Quanitative
	Product ion (daughter) scan	Mass filter	Active	Scan	Qualitative
	Precursor ion (parent) scan	Scan	Active	Mass filter	Usually Qualitative, sometimes Quanitative
	Multiple reaction monitoring (MRM)	Mass filter	Active	Mass filter	Quanitative
	Neutral loss	Scan	Active	Scan	Usually Qualitative, sometimes Quanitative

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In



Chapter Three

IntelliStart[™] Console

MS Console



- Xevo TQ-S micro functions
 - IntelliStart
 - Manual Optimization
 - MS Display
 - Interactive Fluidics
- Sample Tune and Develop Method
- Instrument Setup and Calibration

MS Console



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Xevo TQ-S micro Console View



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Xevo TQ-S micro – IntelliStart



Xevo TQ-S micro – Manual Optimization

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Xevo TQ-S micro – MS Display



Xevo TQ-S micro Interactive Fluidics

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Xevo TQ-S micro – Interactive Fluidics





Console Control Functions





Console Log Entries

System Guatemary Solvert Manager Guatemary Solvert Manager Guatemary Solvert Manager Source Configure Manual Optimization MS Display Interactive Fluidics Plots Maintenance Counters Logs	Console (Local) - [Xevo TQ-Smi	cro MS Detector]				
Contraction of the second seco	Console (Local) - [Xevo TQ-Smin Cuatemary Solvent Manager Column Column Xevo TQ-Smicro MS Detector IntelliStat Manual Optimization MS Display Interactive Fluidics Plots Maintenance Counters Logs Create Log Entry Applies to: XEVO-TQSmicro#QEAR	Control Configure Maintain Troubl Create log of status Ion Mode ES- Flow Path Source Temperature Desolvation Temperature Cone Gas Flow Desolvation Gas Flow	eshoot Help entry Waste 148 °C 596 °C 0 L/Hr 994 L/Hr	settings Tune System ESI NEG 201 Calibration Calibration_2014121	4Dec1 0 when the	Power Status
Comment: Sample Cone Cleaned Sample Cone Cleaned Image: Comment: Image: Comm	Comment: Sample Cone Cleaned	ОКСа	e eratur eratur -7.0	- ил		In the second se

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Console Log Entries

Console (Local) - [Logs]							X
System Quatemary Solvent Manager Sample Manager FTN Column	Control Configure Main	ain Troubleshoot H Content:	lelp System	or Module:	2		
Xevo TQ-Smicro MS Detector IntelliStart	All	User log entries	Current	System •	•		
Manual Optimization	records						U
MS Display Interactive Fluidics	Date and Time	Operator 1	Instrument	Comment			Refresh
Plots Mainte ance Counters							e
Logs						-	Print
							Home
System Status							
	details of current recor	d					
						*	
						-	
	*						14

Console Troubleshooting Functions



IntelliStart



- IntelliStart is designed to automatically monitor instrument readiness.
- Where possible, the system may also take corrective action to rectify any failed system checks
- IntelliStart comprises:
 - Automated system checking software
 - Diagnostic electronics
 - Integrated fluidics device
 - Three built-in vial locations for set-up/user solutions

Using IntelliStart to Monitor the Xevo TQ-S micro

🎬 MassLynx - Default - test.SPL _ = × -1 Eile Yiew Run Help 😂 🔹 📄 🚵 🛃 🎒 🕨 🔲 🔢 🖉 Shortcut 🗟 Queue 🐼 Status Queue Is Empty Spectrum Chromatogram Map Edit - Samples -Instrument @ ent File Name Sample ID File Text MS File Inlet File Bottle Inject Volume Sample Type Conc A Control 1 test_001 test_MRM_su... xevo_train_001 1:7 5,000 Instru 2 vba091_pos_.. vba091_pos_... xevo_train_20... 1:1 0.000 3 vba091_pos_... vba091_pos_... xevo_train_20... 1:1 0.000 Inlet Method Tools 4 vba091_pos_... vba091_pos_... xevo_train_20... 1:1 0.000 5 vba091_pos_... vba091_pos_... xevo_train_20... 1:1 0.000 6 vba091_pos_... vba091_pos_... xevo_train_20... 1:2 0.000 OpenLynx Solvent Monitor TTTT FargetLynx MS Method $\mathcal{\Lambda}$ MS Tune MS Console F/J Edit Shutdown or Startup J Shutdown Startup Options System Status telliStar1 Mot Ready Instrument in standby Ready Not Scanning 0:0 Only Error Shutdown Enabled 1

Using IntelliStart to Resolve "Not Ready" Conditions

A ACQUITY UPLC Console (Local) - [Xevo TQ-S MS Detector IntelliStart] G ACQUITY UPLC System Control Configure Maintain Troubleshoot Help 🤪 Power 🛛 😛 Operate 🗄 Binary Solvent Manager Instrument in standby 🗄 Sample Manager E Column E Xevo TQ-S MS Detector IntelliStart R IntelliStart Manual Optimization MS Display The detector must be in Operate mode to acquire data. The detector might have been Interactive Fluidics put in Standby manually, or a detector error could have occurred. To attempt to switch Plots the detector to Operate mode with IntelliStart, click Resolve. Maintenance Counters Logs Resolve Control Contro Ion Mode: ES+ Type of Analysis: Quantitative ~ Show Instrument set-up options Development ES+ 100 Sample Tune and Develop Method Man. ES-Checks LC/MS System Check has) System Status 0 Xevo TQ-5 MS Detector: Instrument in standby Tune

Using IntelliStart to Resolve "Not Ready" Conditions

A ACQUITY UPLC Console (Local) - [Xevo TQ-S MS Detector IntelliStart] G ACQUITY UPLC System Control Configure Maintain Troubleshoot Help Power Operate 🕀 Binary Solvent Manager 🗄 Sample Manager 🕀 Column E Xevo TQ-S MS Detector IntelliStart Stop Flow Home Rome Stop Stop Stop Coperate Operate API IntelliStart Manual Optimization MS Display Interactive Fluidics Plots Maintenance Counters Logs Ion Mode: ES+ Type of Analysis: Quantitative Y Setting gas and waiting for settle Show Instrument set-up options Development ES+ lan, ** Sample Tune and Develop Method ES-Checks hall? LC/MS System Check System Status

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IntelliStart Configuration



IntelliStart Configuration

IntelliStart Configuration IntelliStart ~ Checks Exhaust problem warning Source heater disconnected 4 **Desolvation heater disconnected** ✓ Operate Source temperature settling failure Desolvation temperature settling failure Probe temperature settling failure Source temperature settling Desolvation gas flow Desolvation temperature settling Probe temperature settling Service due 1 Fluidics leak detected Pump override enabled Project path accessible Disk space Y I c /Mar company characteristic have a sufficient of Properties OK. Cancel

IntelliStart Configuration Properties

IntelliStart Chec	k Properties
Name:	Calibration expiry
Message:	Calibration expired
Console text:	The calibration has expired. To renew the calibration, click Resolve to perform an instrument setup check. If the check fails then a full instrument setup will begin, followed by a sample tune.
Period / secs:	3.0
Action on start:	Warn
Action on error:	No action
Test:	Maximum days since check date 180 days
Mandatory:	No
Log on error:	Yes
	OK Cancel

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IntelliStart Functions

- Instrument Set-up
 - Instrument Resolution
 - Instrument Calibration
- Development
 - Sample Tune
 - Develop Method
- Checks
 - LC/MS System Check

Xevo TQ-S micro IntelliStart Function Flowchart



When the instrument is installed, the engineer will create a valid calibration for each of the four analysis types. Recalibrate the instrument as required.



Sample Tune and Develop Method

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Using IntelliStart for Sample Tuning.



Sample Tune and Develop Method

A ACQUITY UPLC Console (Local) - [Xevo TQ-S MS Detector IntelliStart] B ACQUITY UPLC System Control Configure Maintain Troubleshoot Help Power 😔 Operate 🗄 Binary Solvent Manager 🗄 Sample Manager 🕀 Column E Xevo TQ-S MS Detector IntelliStart IntelliStart Manual Optimization MS Display Home Home Constant Operate Constant April Interactive Fluidics Plots Maintenance Counters Logs Ion Mode: ES+ Type of Analysis: Quantitative × Show Instrument set-up options Development ES+ Sample Tune and Develop Method ES-System Status Checks hall * LC/MS System Check Tune

Sample Tune and Develop Method – Basic Mode

IntelliStart Setup Parameters IntelliStart Sample Tune and Develop Method 10 **Compound Details Compound Name** Molecular Mass/Formula Alprazolam 308.2 AlprazolamD5 313,2 Reserpine 608.3 Terfenadine 471.3 Multiply Charged Parents 0 Method Details O Create New Sample Tune ○ Load Existing Sample Tune Sample Tune Name: quantify.ipr Invoke Manual Optimisation Develop SIR method: alprazolam.exp Export To LC/MS System Check Develop MRM method: alprazolam.exp Append to existing methods alprazolam Print Report Save Report As: Daughter ion settings Number of MRM transitions per compound: 2 × 0) Fluidics ~ <u>A</u> Sample FlowRate: 10.0 Flow Path: Combined Sample Reservoir: B Y Help Switch to advanced mode Start Save Cancel

Sample Tune & Develop Method – In Progress

A ACQUITY UPLC Console (Local) - [Xevo TQ MS Detector IntelliStart] ACQUITY UPLC System Control Configure Maintain Troubleshoot Help Power 😔 Operate 🕀 Binary Solvent Manager 🗄 Sample Manager 🕀 Column E Xevo TQ MS Detector IntelliStart IntelliStart Manual Optimization Stop Flow Home Resolve Construction Stop Operate Construction API MS Display Interactive Fluidics Plots Maintenance Counters Logs Ion Mode: ES+ Type of Analysis: Quantitative × Ion mode = ES+ : Searching for daughters... Show Instrument set-up options Development ES+ ES-System Status Sample Tune and Develop Method Checks LC/MS System Check has Tune

Sample Tune & Develop Method – Complete

A ACQUITY UPLC Console (Local) - [Xevo TQ MS Detector IntelliStart] ACQUITY UPLC System Control Configure Maintain Troubleshoot Help Power 📀 Operate 🗄 Binary Solvent Manager 🗄 Sample Manager E Column E Xevo TQ MS Detector IntelliStart Stop Flov Home Resolve Start Operate Operate IntelliStart Manual Optimization MS Display Interactive Fluidics Plots Maintenance Counters Logs Ion Mode: ES+ Type of Analysis: Quantitative × Show Instrument set-up options Development 🖌 ES+ System Status 100 Sample Tune and Develop Method ES-Checks 20 LC/MS System Check has Tune

Sample Tune & Develop Method – Report



Sample Tune & Develop Method – Advanced Mode

- Advanced Sample Tune Options
- System Setup Using Intellistart

ple T	une and Develop Method					
Iom	pound Details					
	Compound Name	Molecular Mass/Formu	Ja Adduct A+	Adduct B+	Adduct A-	Adduct B-
	Alprazolam	308.2	[M+H]+ 💌	V	~	V
~	AlprazolamD5	313.2	[M+H]+ 💟	~	~	Y
	Reserpine	608.3	[M+H]+ 💌	~	~	Y
	Terfenadine	471.3	[M+H]+ 💌	Y	~	Y
Sav	Develop SIR method: Develop MRM method: ve Report As:	alprazolam.exp alprazolam.exp alprazolam		Expor	rt To LC/MS Sy nd to existing Report	stem Check methods
)ptii Coni Colli	mization Ranges e Voltage: Default (2 - ision Energy: Default (2 -	100) V V 80) V eV	ghter ion settings uber of MRM transitions est Fragment Ion Mass: F	per compound ; Exclude Losses	1: 2 120.0	V 🕑
the state	lics					

Sample Tune & Develop Method – Custom Cone Voltage

IntelliStart Setup Parameters IntelliStart Sample Tune and Develop Method 40 Compound Details **Compound Name** Molecular Mass/Formula Adduct A+ Adduct B+ Adduct A-Adduct B-Alprazolam 308.2 [M+H]+ 💌 × V Y AlprazolamD5 313.2 [M+H]+ 💌 V ~ Y Reserpine 608.3 × × Y [M+H]+ ¥ Y ¥ Terfenadine 471.3 [M+H]+ 💌 Y Multiply Charged Parents 0 Method Details **Custom Cone Voltage** O Load Existing Sample Tune Create New Sample Tune quantify.ipr Sample Tune Name: Cone Voltage Range alprazolam.exp Develop SIR method: * Save Delete Name: ✓ Develop MRM method: alprazolam.exp Start Cone Voltage: alprazolam Save Report As: End Cone Voltage: 100 **Optimization Ranges** Daughte Number of MRM transitions per compound: 2 × 0 V Y Default (2 - 100) Cone Voltage: 120.0 Da Lowest Fragment Ion Mass: V eV Collision Energy: Default (2 - 80) ustom Collision Energy **Collision Energy Range** Fluidics Save Delete Name: Sample Reser Flow Path: Combined ~ Start Energy: eV End Energy: eV Help Switch to basic mode

Sample Tune & Develop Method – Exclude Losses Options

IntelliStart Setup Parameters **Exclude Losses** Inte Exclude Losses Sample Tune and Develop Method Losses to exclude: **Custom Losses:** CO2 (44) <-**Compound Details** Water (18) -> **Compound Name** Molecular Mass/Form ✓ Alprazolam 308.2 AlprazolamD5 Custom 313.2 Reserpine 608.3 Terfenadine 471.3 Multiply Charged Parents **Method Details** • Create New Sample Tune O Load Existing Sample quantify.ipr Sample Tune Name: Develop SIR method: alprazolam.exp Develop MRM method: alprazolam.exp alprazolam Save Report As: OK Defaults Cancel **Optimization Ranges** Da Cone Voltage: Default (2 - 100) V ¥ 120.0 Da Lowest Fragment Ion Mass: Collision Energy: Default (2 - 80) V eV Exclude Losses... Fluidics ⚠ Sample Reservoir: B * Sample Flow Rate: 10.0 Flow Path: ~ Combined Help Switch to basic mode Start Save Cancel

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Chapter Four

Data Acquisition

MassLynx Overview

- MassLynx Structure
- Automated data acquisition
 - Projects
 - Creating a Sample list
 - Queue properties
 - Real time data
 - Shutdown methods



MassLynx Architecture - Main Window



Project Sub-Folders

- Acqudb Acquisition files
 - Tune files (.ipr)
 - MS (instrument) calibration files (.cal)
 - LC method files (.wat or other)
 - MS method files (. exp)
- Curvedb Quantify calibration curves (.cdb)
- Data Raw data files (.raw)
- Methdb Quantify methods (.mdb)
- Peakdb Peak lists (.pdb)
- Sampledb Sample lists (.spl)







Creating a Sample List



- Open an existing project OR
- Create a new project
- Build the sample list
 - File Name
 - MS File
 - Inlet (LC) file
 - Bottle (sample location)
 - Injection Volume

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Open Existing Project



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🏆 MassLynx - Diverse - diverse.SPL View Run Help 🗃 Open Project... E Project Wizard... Open <u>D</u>ata File... New Ctrl+N 🔁 Open... Ctrl+O Save Ctrl+S × **Create Project** Save As... Sample List Properties... drugmixture Project name Import Worksheet... Import Data... drug related analyses Description 🞒 Print... Ctrl+P C:\MassLynx\ Location Browse Print Setup... Exit Quan < Back Next > Cancel

Create New Project

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Project Wizard

Create Project		Acqudb and copied fro project to	nd Methdb fo m the existin the new proj	olders are ig or current ect. Sampledb				
🚫 Create new project		only if sele	ected					
Create using current	project as tem	plate						
Copy sample lists								
Create using existing	project as tem	nplate						
Existing project C:	\MassLynx\Div	verse.PRO	Browse					
		< Back	Finish	Cancel				

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New Sample List



Customize Sample List Format


Changing Column Properties

🏆 Ma	assLynx - drugmixture - unt	itled								
<u>F</u> ile	<u>V</u> iew <u>R</u> un <u>H</u> elp									
• • • • • • • • • • • • •										
	Queue Is Empty									
ц	Instrument @ Spectrum Chromatogram Map Edit - Samples -									
me	0	File	Name File Text	MS File	Inlet File	Bottle Inject Volume				
tru		1				Cut				
Ins	Inlet Method					Paste				
S	(BR)					Add				
T ₀						Insert				
a	Solvent Monitor					Clear Selected				
miz	TTTT					Properties				
Opti	MS Method					Remove Column				
an(Field Pr	operties				Customize Display				
0n O	Field ID	SAM		[ОК	AutoSampler Bed Layout				
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Saving the Sample List Format

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Create an MS Method

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Editing the MS Method

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	Scan 📝 Parents 📝 Daughters 📝 Neutral Loss 📝 Survey
Points Per Peak: [13:704	
Total Run Time: 4.00	
No. Type	Information Time
1 def MRM of 2 mass pairs, Time 0	0.00 to 4.00, ES+
	Function: 1 MRM
	Method
	Ionization Mode ES+ V Compound Name Parent (m/z) Daughter (m/z) Auto Dwell (s) Cone (V) Collision (V) PIC Comments
	I Alprazolam 208.2 281 0.05 40 25 1
	2 Aprazolamus 314.2 200.1 0.05 40 25
	Use Tune Cone Voltage
	Use Tune Collision Energy
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Waters[™]

Saving the MS Method



Select MS Method

7 M	lassLynx - drugmixture - unt	titled									
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Selecting the ACQUITY Method

🍸 MassLynx - drugmixture - untitled File View Run Help 2 📃 🔢 🖉 Shortcut 🗟 Queue 🐼 Status • **Queue Is Empty** Spectrum Chromatogram Map Edit - Samples -Instrument Instrument @ File Name Inlet File File Text MS File Vial Inject Volume Alprazolam_Std_1 Alprazolam 0.000 1 * Inlet Method Tools **Inlet Methods** 5_95_Amm_MeOH_2mins_4_mins Alprazolam Solvent Monitor Alprazolam_4min QuanOptimize OK Cancel ารักา MS Method

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Adding Samples to the Sample List

7 M	assLynx - drugmixture - unt	titled					
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ne		File Name	File Text	MS File	Inlet File	Vial	Inject Volume
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Complete Sample List

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nei			File Name	File Text	MS File	Inlet File	Vial	Inject Volume
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	Iniet Method	3	alprazolam_std_3		Alprazolam	Alprazolam_4min	1:3	10.000
Is	Solvent Monitor	4	alprazolam_std_4		Alprazolam	Alprazolam_4min	1:4	10.000
loo		5	alprazolam_std_5		Alprazolam	Alprazolam_4min	1:5	10.000
1		6	alprazolam_std_6		Alprazolam	Alprazolam_4min	1:6	10.000
ize		7	alprazolam_std_7		Alprazolam	Alprazolam_4min	1:7	10.000
E.	10-0-0	8	alprazolam_std_8		Alprazolam	Alprazolam_4min	1:8	10.000
Opt	MS Method	9	alprazolam_std_9		Alprazolam	Alprazolam_4min	1:9	10.000
an(10	alprazolam_std_10		Alprazolam	Alprazolam_4min	1:10	10.000
Qu		11	alprazolam_std_11		Alprazolam	Alprazolam_4min	1:11	10.000
	MS Tune	12	alprazolam_std_12		Alprazolam	Alprazolam_4min	1:12	10.000
í,	no rune	13	alprazolam_std_13		Alprazolam	Alprazolam_4min	1:13	10.000
L		14	alprazolam_std_14		Alprazolam	Alprazolam_4min	1:14	10.000
Ope	MS Console	15	alprazolam_std_15		Alprazolam	Alprazolam_4min	1:15	10.000

Starting the Sample List

<u>File View Run Help</u>	
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	Outou
MassLynx X Save changes to Untitled? Yes No Cancel	Start Sample List Run
	C\MassLynx\drugmixture.PR0
Save As Save in: SampleDB	Pre-Run Samples ⊘Acquire Sample Data I △Auto Process Samples QCMonitor △Auto Quantify Samples Enabled Post-Run Scheduling Priority Nickt Time Desses

Sample List in Progress

File	<u>V</u> iew <u>R</u> un <u>H</u> elp										
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Pro		2	alprazolam_std_2	1	Alprazolam	Alprazolam_4min	1:2	10.000			
		3	alprazolam_std_3		Alprazolam	Alprazolam_4min	1:3	10.000			
		4	alprazolam_std_4		Alprazolam	Alprazolam_4min	1:4	10.000			
		5	alprazolam_std_5		Alprazolam	Alprazolam_4min	1:5	10.000			
		6	alprazolam_std_6		Alprazolam	Alprazolam_4min	1:6	10.000			
		7	alprazolam_std_7		Alprazolam	Alprazolam_4min	1:7	10.000			
		8	alprazolam_std_8		Alprazolam	Alprazolam_4min	1:8	10.000			
		9	alprazolam_std_9		Alprazolam	Alprazolam_4min	1:9	10.000			
		10	alprazolam_std_10		Alprazolam	Alprazolam_4min	1:10	10.000			
		11	alprazolam_std_11		Alprazolam	Alprazolam_4min	1:11	10.000			
		12	alprazolam_std_12		Alprazolam	Alprazolam_4min	1:12	10.000			
		13	alprazolam_std_13		Alprazolam	Alprazolam_4min	1:13	10.000			
		14	alprazolam_std_14		Alprazolam	Alprazolam_4min	1:14	10.000			
		15	alprazolam_std_15		Alprazolam	Alprazolam_4min	1:15	10.000			

Real Time Data Viewing



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Automatic Shutdown

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0015		Enable startup before batch Browse	2)
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otimize	بی این این این این این این این این این ای	Shutdown if queue is in pause	
2 D	MS Method	Shutdown time after batch or error (mine): 10 Configure error shutdown	
Qua		Optimization E-mail on Error Shutdown	
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etLynx	Edit Shutdown or		
arge	Startup		

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ic Shutdown				Waters™
Enable shutdown after batch C:\MassLynx\ShutDown\ShutDownES	_ACE.acl Browse			
Shutdown if queue is in pause				
Shutdown Time Shutdown time after batch or error (mins): 10 Configu	re error shutdown			
Optimization E-mail on Error Shutdown Optimize E-mail recipient				
Shutdown On Error Confi	guration		×	n
Error Shutdown				
MS error	C:\MassLynx\Shutdown\ShutDownES	Browse Gas Thresholds	Shutdown immediately	
MS Comms error		Browse	Shutdown immediately	
Inlet fatal error		Browse	Shutdown immediately	
Ext. device error		Browse Configure CCs	Stutdown immediately	
Solvent Monitor Warning		Browse	St utdown immediately	
Solvent Monitor Acute		Browse	Statdown immediately	
QCMonitor	C:\MassLynx\Shutdown\ShutDownE8	Brows Gas Threshold	<u> </u>	
		Source Gas Thresh	100 (L/Hr)	
			OK Cancel	

Automati

Shutdown Tasks

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down Auto Control Tasks				
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LC Pump Off 🛛 🛛 💽	Task	Pre Delay	Post Delay	Ion Mo
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e Puise o (ms)	<	100	1	>



Chapter Five

Programming Instrument Methods

Programming Instrument Methods

Waters[™]

- Scanning Methods
- Product (daughter) Ion Scan
- Precursor (parent) Ion Scan
- Multiple Reaction Monitoring
- Neutral Loss
- Survey Scan

Accessing MS Method Editor

Waters[™]



MS Method Editor

Experiment Setup - c:\masslynx\default.pr	o\acqudb\default.exp	1000	
<u>File Edit View Options Toolbars Fu</u>	nctions <u>H</u> elp		
SIR MRM MSS	can 🛛 🕜 Parents 🛛 📝 Daughters	🛛 📝 Neutral Loss 🛛 🖉 Survey	
Points Per Peak: 36.036	7		
Total Run Time: 2.50 🔶		L 1	2mins
No. Туре I	nformation		Time
MRM of 4 mass pairs, PIC , Tin	ne 0.00 to 2.50, ES+		
MS and MS/MS	\backslash		
Modes		Function	
		(double click	
		for details)	
			NUM

RADAR™



- Collision cell is permanently filled with collision gas
- Allows MRM data to be collected in parallel to the collection of spectral MS data.
- No appreciable impact on sensitivity

RADAR

Experiment Setup - c:\masslynx\default.pro\acqudb\default.exp	Carlo Carlo	
<u>File Edit View Options Toolbars Functions Help</u>		
Image: Constraint of the second se	🛛 📝 Neutral Loss 🛛 📝 Survey	
Points Per Peak: 18.433		
Total Run Time: 2.50 🔶	1	2mins
No. Type Information	Time	
1 MRM of 4 mass pairs, PIC , Time 0.00 to 2.50, ES+		
2 MS Scan, Time 0.00 to 2.50, Mass 100.00 to 600.00 ES+		
9		NUM

Data Acquisition – Points Per Peak

Experiment Setup - c:\masslynx\default.pro\acqudb\default.exp	
	Daughters Poutral Loss Survey
No. Type 1 MRM or + mass pairs, rice, rice or or or 2.90, c.31	Time
Experiment Setup - c:\masslynx File Edit View Options Ioo Image: Signal	Points Per Peak Chromatography Peak Width(s) 4 Required Points Per Peak 12 Set As Default OK Cancel Vdefault.pr Ibars Fu MS S

MS Method Editor - Full Scan Function

K Experiment Setup - c:\masslynx\default.pro\acqudb\default.exp File Edit View Options Toolbars Functions Help D 🗳 🖬 🚭 🗹 🗙 📝 MRM 📝 MS Scan U SIR 🕜 Parents 📝 Daughters Neutral Loss 🛛 🖉 Survey Points Per Peak: Total Run Time: 0.00 ++ No. Type Information Time 28 Function:1 MS Scan Mass (m/z) Method 100 Ionization Mode ES+ 🔻 Start 600 End Data Continuum -Time (Mins) Scan Duration (secs) 0 0.1 Start Sca<u>n</u> Time 2.5 End Cone Voltage Probe Temperature 📃 Use Tune Page Use Tune Page Settings Cone Voltage (V) 30 20 Probe Temp Use Cone Voltage Ramp Use Probe Temp Ramp CV Ramp... Probe Temp Ramp... OK Cancel NUM

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Setting the Scan Duration

unction:1 MS S	can	×		
Mass (m/z) <u>S</u> tart Engl	100 600	Method Ionization Mode ES+ D <u>a</u> ta Continuum		
Time (Mins) S <u>t</u> art <u>E</u> nd	0 2.5	Scan Duration (secs) Sca <u>n</u> Time 0.1		
Cone Voltage Use Tune Page Cone Voltage (V) 30 Use Cone Voltage Ramp CV Ramp		Probe Temperature Use Tune Page Settings Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp		

Setting the Cone Voltage

Function:1 MS	Scan		Cone Voltage
Mass (m/z) <u>S</u> tart En <u>d</u>	600	Method Ionization Mode ES+ Data Continuum	Use Tune Page Cone Voltage (V) 30
Time (Mins) S <u>t</u> art <u>E</u> nd	0 2.5	Scan Duration (secs) Sca <u>n</u> Time 0.1	CV Ramp
Cone Voltag Use Tun Cone Voltag	ge ne Page ge (V) 30 ne ⊻oltage Ramp (Ramp	Probe Temperature Use Tune Page Settings Probe Temp 20 Use Probe Iemp Ramp Probe Temp Ramp	Cone Voltage Use Tune Page Cone Voltage (V) 30 Use Cone Voltage Ramp CV Ramp
		OK Cancel	

Setting the Cone Voltage Ramp

Mass (m/z)		Method	
<u>S</u> tart	100	Ionization Mode ES+ 🔻	
End	600	D <u>a</u> ta Continuum 🔻	Ramp Dialog
Time (Mins)		Scan Duration (secs)	Ramp Gradient
-			Start Mass 100
Start	U	Scan_Time U.I	<u>E</u> nd Mass 1500
<u>E</u> nd	2.5		Cone Start Volts 20
Cone Voltac	10	Probe Temperature	Cone End Volts 60
Use Tun	e Page	Willes Tures Page Settings	Gradient (V/Da) 0.036
Cone Voltag	pe (V) 30	Probe Temp 20	Selected voltage ramp OK for entire mass r
🔽 Use Con	e Voltage Ramp	Use Probe <u>T</u> emp Ramp	
CV	Ramp	Probe Temp Ramp	E

Product Ion Analysis (Daughter Scan)

K Experiment Setup - c:\masslynx\default.pro\acqudb\default.exp File Edit View Options Toolbars Functions Help D 🚅 🖬 🚭 🗹 🗙 Ø SIR MRM 📝 MS Scan 📝 Parents 🖉 Daughters Neutral Loss 🛛 🖉 Survey Points Per Peak: Total Run Time: 0.00 ++ X Function:1 Daughter Scan No. Type Inform Time Method Mass (m/z) 292.00 Daughters of Ionization Mode ES+ 💌 50.00 Start Data Continuum ٠ 350.00 End Time (Mins) Scan Duration (secs) Start 0 0.1 Sca<u>n</u> Time 2.5 End Collision Energy Cone Voltage 🔲 Use Tune Page Use Tune Page Collision Energy (V) 25 Cone Voltage (V) 30 📃 Use Collision Energy Ramp 🔲 Use Cone <u>V</u>oltage Ramp CE Ramp... CV Ramp... Probe Temperature Use Tune Page Settings OK Cancel Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp.. NUM

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Mass Range and Collision Energy

ionit buuginer sean		Daughters of	292.00
Mass (m/z) Daughters of 292.00	Method Ionization Mode ES+ -	<u>S</u> tart	50.00
<u>Start</u> 50.00 End 350.00	D <u>a</u> ta <u>Continuum</u>	End	350.00
Time (Mins)	Scan Duration (secs)		
Start 0	Sca <u>n</u> Time 0.1		
<u>End</u> 2.5			
Collision Energy	Cone Voltage		
Collision Energy (V) 25	Cone Voltage (V) 30	Collision Energy	
composition gy (1) 20			
Use Collision Energy Ramp	Use Cone ⊻oltage Ramp	📃 Use Tune Pa	age
Use Collision Energy Ramp	Use Cone ⊻oltage Ramp	Collision Energy	age (V) 25
Use Collision Energy Ramp CE Ramp Probe Temperature	Use Cone <u>V</u> oltage Ramp	Collision Energy	age (V) 25
Use Collision Energy Ramp CE Ramp Probe Temperature Use Tune Page Settings Probe Temp 20	Use Cone Yoltage Ramp	Collision Energy	age (V) 25 Energy Ramp

Precursor Ion Analysis (Parent Scan)

K Experiment Setup - c:\masslynx\default.pro\acqudb\default.exp File Edit View Options Toolbars Functions Help D 🚅 🖬 🚭 🗹 🗙 📝 Daughters Ø SIR MRM 📝 MS Scan 🕜 Parents Neutral Loss 🛛 🖉 Survey Points Per Peak: Total Run Time: 0.00 ++ X Function:1 Parent Scan No. Type Inform Time Mass (m/z) Method 97.00 Parents of ES+ 🔻 Ionization Mode 50.00 Start Data Continuum • 400.00 End Time (Mins) Scan Duration (secs) 0 0.1 Scan Time Start 2.5 End Collision Energy Cone Voltage 🔲 Use Tune Page 🔲 Use Tune Page Collision Energy (V) 25 Cone Voltage (V) 30 📃 Use Collision Energy Ramp 🔲 Use Cone ⊻oltage Ramp CE Ramp... CV Ramp... Probe Temperature 🕡 Use Tune Page Settings OK Cancel 20 Probe Temp Use Probe Temp Ramp Probe Temp Ramp... NUM

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Setting the Mass Range

Mass (m/z) Parents of	97.00	Method Ionization Mode ES+ ▼		
<u>S</u> tart	50.00			
Eng	400.00	D <u>a</u> ta <u>Continuum</u>		
lime (Mins)		Scan Duration (secs)		
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Ind	2.5		Mass (m/z)	
Collision Energy		Cone Voltage	Parents of	97.00
🗐 Use Tune Pa <mark>g</mark> e		🔲 Use Tune Page	Charl	50.00
Collision Energy (V)	25	Cone Voltage (V) 30	<u>s</u> tart	30.00
Use Collision Energ	gy Ramp	Use Cone <u>Voltage</u> Ramp	En <u>d</u>	400.00
Probe Temperature				
🗸 Use Tune Page S	ettings	OK Cancel		
Probe Temp [20			
Use Probe <u>T</u> emp F	Ramp			
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SIR Function

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📝 SIR 📝	MRM 📝 MS Scan 📝 Parents 📝 Daughters 📝 Neutral Loss 📝 Survey
Points Per Peak:	
Total Fun Time: 0.00	↔
No. Type	Information Time
	Function:1 SIR
	Method Channels
	Ionization Mode ES+ Compound Name Mass (m/z) Auto Dwell Dwell (s) Cone (V)
	Span 0 1 Erythromycin 734.3 0.025
	Use Tune Cone Voltage
	Retention Window (Mins)
	Start 0
	<u>E</u> nd 2.5
	Probe Temperature
	Use Tune Page Settings
	Probe Temp
	Use Probe Iemp Ramp
	Probe Temp Ramp Add Delete Clear All Undo Redo Fill Down
	OK Cancel

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Monitored Masses and Dwell Time

X Function:1 SIR Method Channels Ionization Mode ES+ 🔻 **Compound Name** Mass (m/z) Auto Dwell Dwell (s) Cone (V) Erythromycin 734.3 0.025 0 1 Span Use Tune Cone Voltage E Retention Window (Mins) 0 Start 2.5 End Probe Temperature Use Tune Page Settings 20 Probe Temp ٠ (111 Use Probe Temp Ramp Fill Down Add Delete Clear All Undo Redo Probe Temp Ramp... OK Cancel

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Entering Masses

Method		Channe	əls				
Ionization Mode	e ES+ ▼		Compound Name	Mass (m/z)	Auto Dwell	Dwell (s)	Cone (V)
Spa <u>n</u>	0	1	Erythromycin	734.3		0.025	1
Use Tune Cone	Voltage 📃						
Retention Wind	dow (Mins)						
Start	0						
<u>E</u> nd	2.5						
Probe Tempera	ature						
Use Tune Page	e Settings 🛛 💟						
Pro <u>b</u> e Temp	20						
Use Probe <u>T</u> em	p Ramp 🗌	٠		ш)
	amp]	Adi	<u>D</u> elete	jear All Ur	ndo Re	do Fill Dowr	
Probe Temp R							

Multiple Reaction Monitoring

	M X	
🖉 SIR	MRM 🕜 MS Scan 🛛 🖉 Parents 📝 Daughters 🖉 Neutral Loss 🖉 Survey	
Points Per Peak:		
TUD T Ī		
Total Run Time:		
No. Type	Information Time	
	Function:1 MRM	
	Method Channels	
	Iorization Mode ES+ Compound Name Parent (m/z) Daughter (m/z) Auto Dwell Dwell (s) Cone (V) Collision (V) PIC Commercian	its
	Spag 0	
	Use Tune Cone Voltage 📃	
	Use Tune Collision Energy	
	Hetention Window (Mins)	
	End 25	
	Probe Temperature	
	Use Tune Page Settings	
	Use Probe Temp Ramp	
	Probe Temp Ramp	
	PIC Scan	
	PIC Scan Daughter Scan	
	Use Default Threshold	
	Activation Threshold 20	
	Use Default Collision Energy	
1	Collision Energy 20 Add Delete Clear All Undo Redo Fill Down	
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Entering Masses

Notiod	Cha	nnels								
Ionization Mode ES+		Compound Name	Parent (m/z)	Daughter (m/z)	Auto Dwell	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
	1	Acetaminophen	152.07	110.06		0.025	30	16	100	
Spa <u>n</u> O	2	Caffeine	195.09	138.06		0.025	32	19	100	
	3	Sulfadimethoxine	311.08	156.01		0.025	30	20	10	
	4	Verapamil	455.29	169.09		0.025	41	30		
Use Tune Cone Voltage 🛛 🗍	9									
Use Tune Collision Energy	1									
	-									
Hetention Window (Mins)										
Start 0										
End 25										
Probe Temperature										
Use Tune Page Settings										
Probe Temp 20										
Probe Temp 20 Use Probe Temp Ramp										
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp										
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp										
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp PIC Scan										
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp PIC Scan Use Default PIC Scan funct	 pn									
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp PIC Scan Use Default PIC Scan funct PIC Scan Daughter Scan	on T									
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp PIC Scan Use Default PIC Scan funct PIC Scan Daughter Scan Use Default Threshold	on T									
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp PIC Scan Use Default PIC Scan funct PIC Scan Daughter Scan Use Default Threshold Activation Threshold	on T									
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp PIC Scan V Use Default PIC Scan funct PIC Scan Daughter Scan V Use Default Threshold Activation Threshold 20 Minimum Threshold) pn T									
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp PIC Scan Use Default PIC Scan funct PIC Scan Daughter Scan U Use Default Threshold 20 Activation Threshold 20 Minimum Threshold 500000										
Probe Temp 20 Use Probe Temp Ramp Probe Temp Ramp PIC Scan ✓ Use Default PIC Scan funct PIC Scan ✓ Use Default Threshold Activation Threshold Øuinimum Threshold Øuise Default Collision Energy))]	Delay	Create							

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PICS Acquisition Mode

- Acquire quantitative MRM
- Use MRM data as a specific trigger for the acquisition of a product ion spectrum
- This spectral data could provide useful extra information about a suspect MRM quantification result

PICS Example Data: (UPLC/MS/MS of Sulfadimethoxine)



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PICS Example Data: (UPLC/MS/MS of Sulfadimethoxine)

SDM SSB02JUN08_002 MRM Channel ES+ TIC (SDM) 2.37e6 1.01 100-Area Standard MRM 15 data points per peak 0.99 1.01 1.02 1.03 1.04 1.00 1.05 1: MRM SSB02JUN08_009 Channel ES+ TIC (SDM) 2.24e6 1.01 100-31278.297 Area PICS ROALISTION PICS acquisition mode activated 13-14 data points per peak - Time 0.99 1.00 1.01 1.02 1.03 1.04 1.05 ©2025 Waters Corporation

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PICS Example Data: (UPLC/MS/MS of Sulfadimethoxine)





Product Ion Confirmation Setup

			Channel	8								
Ionization Mode	ES+	-		Compound Name	Parent (m/z)	Daughter (m/z)	Auto Dwell	Dwell (s)	Cone (V)	Collision (V)	PIC	Comments
	(construction)		1	Acetaminophen	152.07	110.06		0.025	30	16	100	
Span	0		2	Caffeine	195.09	138.06		0.025	32	19		
			3	Sulfadimethoxine	311.08	156.01		0.025	30	20	J	
			4	Verapamil	455.29	169.09		0.025	41	30		
Use Tune Cone Vol	age											
Jse Tune Collision B	nergy	100										
Retention Window	(Mins)											
		_										
Statt	U											
<u>E</u> nd	2.5											
Probe Temperature												
🔽 Use Tune Page	Settings											
Probe Temp	20											
Use Probe <u>T</u> em	Ramp											
Probe Temp Ramp												
(1000 Temp Tramp	삧											
the second se												
PIC Scan	Scan tur	nction										
PIC Scan V Use Default PIC	e e a i i i a											
PIC Scan V Use Default PIC PIC Scan Daug	nter Scan	Ŧ										
PIC Scan Use Default PIC <u>P</u> IC Scan Daug Use Default Thr	nter Scan	Ŧ										
PIC Scan Use Default PIC PIC Scan Daug Use Default Thr Activation Threshol	nter Scan eshold d 20	×										
PIC Scan Use Default PIC <u>P</u> IC Scan Daug Use Default Thr <u>A</u> ctivation Threshold <u>M</u> inimum Threshold	nter Scan eshold d 20 (500000											
PIC Scan V Use Default PIC PIC Scan Daug V Use Default Thr Activation Threshold Minimum Threshold V Use Default Col	nter Scan eshold d 20 (500000 ision Ene											
PIC Scan Use Default PIC PIC Scan Daug Use Default Thr Activation Threshold Minimum Threshold Use Default Col Collision Energy	nter Scan eshold d 20 500000 ision Ene 20		Add	Delete	Cjear All	Undo Reda	5 Fill D	own				

Product Ion Confirmation Setup

Experiment Setup - c:\masslynx\default.pro\acqudb\default	.exp
File Edit View Options Toolbars Functions Help 	🖉 Daughters 🛛 😰 Neutral Loss 🖉 Survey
Total Run Time: 2. 1 Product Ion Confirmation Settings	0 1 2mins
No. Type Fraction Trigger	Product Ion Confirmation Settings
	Default PIC Settings Default PIC Settings Reset Scan Function Daughter Scan Activation Threshold Level 20 x Background Noise Minimum Activation Threshold 500000 Counts Reset Threshold Level 50 % of Act Threshold Mass above Parent 10 Da Minimum Mass 40 Da Data Continuum Scan Speed 5000 amu/sec PIC Duration 1 secs Collision Energy (V) 20 0K

SIR/MRM Chromatogram Smoothing

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File Edit View Options Toolbars Functions Help Image:	
Configure SIR/MRM smoothing	
Image: Single	
Total Run Time: 2.51 Product Ion Confirmation Settings	nins L
No. Type Fraction Trigger Time	
Points Per Peak Chromatography Peak Width(s) 4 Required Points Per Peak Set As Default OK	
Configure the parameters for SIR/MRM chromatographic smoothing.	NUM

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Neutral Loss

<u>File Edit View Options Toolbars Func</u>	tions <u>H</u> elp		
D 🖻 🖬 🏐 🖉 🗙			
💅 SIR 🛛 📝 MRM 🛛 📝 MS Scal	n 🛛 🕜 Parents 🗍 🕜 Daughte	ers 📝 Neutral Loss 📝 Survey	
Points Per Peak:			
Total Run Time: 000			
	~		
No. Type Inf	Function:2 Neutral Loss	T	ime
	Mass (m/2) Losses of 44.00 Start 100.00 Eng 350.00 Time (Mins) Start 0 End 2.5 Collision Energy Use Tune Page Collision Energy (V) 15 Use Collision Energy Ramp CE Ramp Probe Temperature Use Tune Page Settings Probe Temp 20 Use Probe Iemp Ramp	Ionigation Mode ES+ Dgta Continuum Scan Duration (secs) Scan Time 0.1 Cone Voltage Use Tune Page Cone Voltage (V) 30 Use Cone Voltage Ramp CV Ramp OK Cancel	
	Probe Temp Ramp		NUM

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Setting the Mass Range

Mass (m/z)		Method		
Losses of Start	44.00	Ionization Mode ES+ • Data Continuum •		
En <u>d</u> Time (Mins) Start	0	Scan Duration (secs) Scan Time 0.1	Mass (m/z) Losses of	44.00
<u>E</u> nd	2.5		→ <u>S</u> tart	100.00
Collision Energy	age	Cone Voltage	En <u>d</u>	350.00
Collision Energy	(V) 15 n Energy Ramp amp	Cone Voltage (V) 30		
Probe Tempera Use Tune P Probe Temp Use Probe] Probe Temp R	ture age Settings 20 Cemp Ramp amp	OK Cancel		

Points Per Peak:	
Total Run Time: 0.00 ↔	
No. Type	Inf Function: 1 Survey Scan
	Survey Switch
	Switch From Switch To
	Scan Type MS Scan V Scan Type Daughter Scan
	Method Method
	Data Continuum - Data Continuum -
	Mass (m/z) Mass (m/z)
	Start 100 Start 50
	En <u>d</u> 750 En <u>d</u> 700
	Scan Duration (secs)
	Scan_Time 0.1 Scan_Time .1
	Cone Voltage Collision Energy
	Cone Voltage (V) 30 Collision Energy 1 20
	Collision Energy Collision Energy 2 30
	Time (Mins) Start 0 End 2.5

Survey Scan Method Editor

😰 Experiment Setup - c:\masslynx\default.pro\acqudb\default.exp

File Edit View Options Toolbars Functions Help

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Survey Scan Method Editor: Survey Tab

× Function:1 Survey Scan Survey Switch Switch From Switch To Scan Type Daughter Scan Scan Type MS Scan -Method Method Ionization Mode Ionization Mode ES+ 🔻 ES+ 🔻 Data Continuum Data Continuum -• Mass (m/z) Mass (m/z) 100 50 Start Start End 750 End 700 Scan Duration (secs) Scan Duration (secs) .1 0.1 Scan Time Scan Time Cone Voltage Collision Energy Cone Voltage (V) 30 Collision Energy 1 20 Collision Energy Collision Energy 2 30 Collision Energy 4 Time (Mins) 0 2.5 Start End OK Cancel Help

Survey Scan Method Editor: Switch Tab

X Function:1 Survey Scan Survey Switch Include Activation Criteria Trigger Criteria Trigger Activation Delay (Mins) 0 Trigger Sensitivity Medium 👻 5 Trigger Threshold Max. Masses of interest per survey scan 2 Resume Criteria 2 Total Time in Switched Scan Mode (s) Detected Precursor Inclusion 3 Re-include after Time (s) Exclusion Window +/- (Da) 0.5 3 Isotope Cluster Range (Da) Specific Mass Selection V Include Masses Retention Time Window +/- (s) 1 Exclude Masses OK Cancel Help

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Survey Scan Method Editor: Include Tab

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Survey Swit	ch Include					
Include Mas	ses					
lon Selec	ction					
🔘 Inclu	ded Masses only					
Inclu	ded Masses Take Prio	rity				
Range S	election					
Range	Masses From	100	-			
in the second		100				
	Masses To	600				
027 200	23 - 1221				Include Masses	
Specific	Mass Selection				Add/Modify	
File				Browse	Include Mass (m/z)	391
		Post and the	Cours		Retention Time (min)	3.0
	Mass Retention	lime	Save		7	
			Save As			
			Add			
			Delete			
			Delete			
			New			
loolude	Maee Window +/ (Da)	0.5	1			
Include	Mass Window +/- (Da)	0.0				
					ОК	Cancel

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Survey Scan Method Editor: Exclude Tab

Range	Masses From	100			
	Masses To	600			
Spe <mark>c</mark> ific M	ass Selection				n'
File				Browse]
	Mass Retention Ti	me	Save		
			Save As]	
			Add		
			Delete]	
			New		
Exclude N	lass Window +/- (Da)	0.5			
Adduct Ma	ass Utilisation				
Exclud	e Adducts of Include	Masses			

Survey Scan Method Editor: Adduct Tab

Adduct Masses Specific Mass	Adduct Mass	Browse Save Save As	
Specific Mass	Adduct Mass 22.0000	Browse Save Save As	
	Adduct Mass 22.0000	Browse Save Save As	
	Adduct Mass 22.0000	Save Save As	
	Adduct Mass 22.0000	Save As	
	22.0000	Save As	
		Add	
		Delate	
		New	
Note: Exclus	ion adducts will only be ge	nerated from the Include specific mass list.	
		Adduct Mass	
		Add/Modify	
		Step Size (m/z) 22	
		OK Cancel	
			8
		<u>2 </u>	

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MS Method Events

🖄 Experiment Setur	p - c:\masslynx\default.pro\acc	udb\default.exp		100		
File Edit View	Options Toolbars Function Configure SIR/MRM smo Configure SIR/MRM sorti Points Per Peak Method events Product Ion Confirmation	ns Help othing ng n Settings	Daughters 🗹	Neutral Loss 🌌 Surve	sy	
No. Type	Fraction Trigger				Time	
Method events Events Time / Mins 0.00 Image:	Event Action Stop flow On Stop flow Switch 2 Switch 3 Switch 4 Infusion Injection Flow State Refil Reservoir Flow Rate Refil Reservoir Softerst Pelsy Corona Current Cone Gas Corona Change Delete Clear Alt	Initial Settings Stop flow No Change Switch 2 No Change Switch 3 No Change Switch 4 No Change Switch 4 No Change Infusion No Change Flow State LC Flow Rate µl/min 5.0 Reservoir No Action Refil No Action Refil No Action Solvent Delay Options API Probe Temperature C 20		Method events Events Time / Mins Event 0.20 Flow State 0.20 Flow State Add Change Du	Action LC LC LC LC LC LC LC LC LC L	Initial Settings Stop flow No Change V Switch 2 No Change V Switch 3 No Change V Switch 4 No Change V Switch 4 No Change V Flow State Vaste V Flow State Vaste V Flow Rate µL/min 5.0 Reservoir No Action V Refill No Action V Volume µL 200 V Solvent Delay Options API Probe C 20
Edit the		OK Cancel		Enable		OK Cancel



Chapter Six

Data Processing

Data Processing

- Displaying and Processing Chromatograms
- ApexTrack[™] Peak Integration
- Integrating and Editing Peaks
- Signal-to-Noise
- Displaying and Processing Spectra

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Creating a Chromatogram

77 M	assLynx - Quantify - Quantii	fy.spl	1							
<u>F</u> ile	<u>V</u> iew <u>R</u> un <u>H</u> elp									
2	•		II 🖉 sh	iortcut 🔒 Q	ueue 🛛 💁 Sta	tus				
				Q	ueue Is	Empty				
×	QuanLynx @	Sp	ectrum	Chromato	gram Ma	ap Edit	- Samp	les -		
L <u>Y</u>			File Name	Sample to	rie rext	MS File	Inlet File	Bottle	Inject Volume	Sample
Jet	50	1	ASSAY01	ID	plasma blank	DEFAULT	DEFAULT	1	10.000	Blank
arg	Edit Mathad	2	ASSAY02	ID2	0.2pg/ml std	DEFAULT	DEFAULT	2	10.000	Standard
-	Luit Methou	3	ASSAY03	ID3	0.5pg/ml std	DEFAULT	DEFAULT	3	10.000	Standard
Xu	× ste	4	ASSAY04	ID4	0.75pg/ml std	DEFAULT	DEFAULT	4	10.000	Standard
LY	4 25	5	ASSAY05	ID5	1pg/ml std	DEFAULT	DEFAULT	5	10.000	Standard
Bio	Process Samples	6	ASSAY06	ID6	2pg/ml std	DEFAULT	DEFAULT	6	10.000	Standard
~		7	ASSAY07	ID7	5pg/ml std	DEFAULT	DEFAULT	7	10.000	Standard
λu)	Q	8	ASSAY08	ID8	10pg/ml std	DEFAULT	DEFAULT	8	10.000	Standard
F	View Results	9	ASSAY09	ID9	15pg/ml std	DEFAULT	DEFAULT	9	10.000	Standard
Sua		10	ASSAY10	ID10	0.3pg/ml QC	DEFAULT	DEFAULT	10	10.000	QC
0		11	ASSAY11	ID11	2pg/ml QC	DEFAULT	DEFAULT	11	10.000	QC
Xu		12	ASSAY12	ID12	12pg/ml QC	DEFAULT	DEFAULT	12	10.000	QC
aLy		13	ASSAY13	ID13	Rat sample 01	DEFAULT	DEFAULT	13	10.000	Blank
Ĕ		14	ASSAY14	ID14	Rat sample 02	DEFAULT	DEFAULT	14	10.000	Analyte
hro		15	ASSAY15	ID15	Rat sample 03	DEFAULT	DEFAULT	15	10.000	Analyte
0		16	ASSAY16	ID16	Rat sample 04	DEFAULT	DEFAULT	16	10.000	Analyte
×		17	ASSAY17	ID17	Rat sample 05	DEFAULT	DEFAULT	17	10.000	Analyte
L		18	ASSAY18	ID18	Rat sample 06	DEFAULT	DEFAULT	18	10.000	Analyte
len		19	ASSAY19	ID19	Rat sample 07	DEFAULT	DEFAULT	19	10.000	Analyte
			1001100	10.00	-				10.000	140 Y 201



Zooming Functions



Extracting Data from Ion Chromatograms

🧱 Chromatogram - [ASSAY07] - 8 × File Edit Display Process Window Tools Help Link A B 📾 🖻 🖻 🐔 丛 🚣 🌑 A 🖷 🛍 🗘 Q' Q' Q' 110 🛊 B ۵ 🗢 5pg/ml std ASSAY07 MRM of 3 Channels AP+ 288.1 > 58 2.81 1.46e4 Mass Chromatogram File: ASSAY07 OK 8-Description (chan): Cancel Ch1.Ch2.Ch3 File... Function: MRM of 3 Channels AP+ * Select All 0 1:274.1 > 182.1 Channels: 2.00 2.20 2.40 2.60 2:288.1 > 58 3:294.1 > 64 Add trace O Replace trace O New window





Channel Selection Results

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Deleting a Chromatogram

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Changing Trace Order

Figure 3

Figure 4

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Restoring Full View of TIC



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Display Chromatograms from Different Runs



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Align Chromatogram Time



Altering Display Properties

Chromatogram - [ASSAY07] File Edit Display Process Window Tools Help - 8 × Z A D : Mass...) A 📲 能 🖄 Q⁺ Q⁻ Q[×] TIC 🛊 🛊 💥 Tic... 5pg/ml std Analog... ASSAY07 MRM of 3 Channels AP+ Remove... TIC 2.81 2.09e4 Real-Time Update ChroTool... DDATool... Range • **Chromatogram Display View** Pointer View.... Normalize Data To: Style Peak Annotation... Largest Peak Customize Toolbar... Verlay Graphs Graph Header 0 Intensity ✓ Toolbar Fill Trace Process Description ✓ Status bar Normalise to Summed Trace Fill Detected Peaks Component Table %-Move To Last O Baseline at Zero Peak List Move To First OBaseline abs 0 Traces . 0 OBaseline % Lowest Point Link Vertical Axes Split Axis 1 v Overlay Step (%) 10 Axis Label Grid Off Horizontal Axis × v Time OK Cancel Header...

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Link Vertical Axes



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Adding Header Information



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× **Chromatogram Display View** Normalize Data To: Style Largest Peak Overlay Graphs Graph Header 0 Intensity Fill Trace Process Description Normalise to Summed Trace Fill Detected Peaks Component Table Baseline at Zero Peak List 0 O Baseline abs OBaseline % 0 O Lowest Point Link Vertical Axes Split Axis 1 10 Overlay Step (%) Axis Label Grid Horizontal Axis Off v Time Y Header... OK Cancel

Adding Header Information

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eader Editor (ChrHeade	er)			×		
Header areas			OK Cancel Clear All			
Cell : Line 1, Left Group RawFileHeader	1		Format			
Element		Format	Header Editor (ChrH	eader)		
RawDataDate RawDataTime SignatureUser SignatureDate SignatureReason Job Task UserName Laboratory Instrument	Add -> <- Remove <- Clear	RawFileHeader,Sample	Header areas			Clear All
			RawFileHeader	~		Format
			Element		Format	
			RawDataDate RawDataTime SignatureUser SignatureDate SignatureReason Job Task UserName Laboratory Instrument	 Add -> <- Remove <- Clear 	End	

Adding Header Information

Adding Header Information

Header Editor (ChrHeader) Header areas OK Cancel Clear All Cell : Line 1, Right Group RawFileHeader Format. v Format Element RawDataDate RawFileHeader,RawDataDate ~ RawDataTime End Add -> SignatureUser SignatureDate <- Remove SignatureReason Job Task <- Clear UserName Laboratory Instrument

Adding Header Information

_ 7 × Chromatogram - [13 analgesic mix faster] File Edit Display Process Window Tools Help - 8 × 30 mm col 02-Mar-2006 13 analgesic mix faster 3: Diode Array Range: 8.442e-1 0.56 0.50 0.64 4.0e-1-Chromatogram Display View AU 2.0e-1-Normalize Data To: Style Largest Peak Overlay Graphs Graph Header 0 00 O Intensity 0 Fill Trace Process Description 1.00 1.10 90 Normalise to Summed Trace 13 analgesic mix faster Fill Detected Peaks 2: Scan ES-Component Table TIC Baseline at Zero 100-Peak List 9.92e6 O Baseline abs 0 OBaseline % 0 2 O Lowest Point Link Vertical Axes Split Axis 0.07 0.09 0.94 Anon Overlay Step (%) Axis Label 1.10 1.00 90 Grid Off Horizontal Axis Time * 13 analgesic mix faster 1: Scan ES+ TIC 100-1.90e8 OK. Cancel Header... 0.57 0.50 0.31 0.12 0.78 0 - Time 0.10 0.20 0.30 0.50 0.80 1.10 0.40 0.60 0.70 0.90 1.00





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Chromatogram Processing

- Smoothing
- Background (Baseline) Subtraction
- Integration
 - Setting integration parameters
 - Baseline Defined Peak Integration
 - Apex Track Peak Integration
 - Integrating a selected chromatogram region
 - Editing detected peaks
 - Peak annotation
- Signal-to-Noise

Smoothing a Chromatogram



Smoothing a Chromatogram



Smoothing a Chromatogram





🔤 Chromatogram - [13 analgesic mix faster] _ 7 > File Edit Display Process Window Tools Help - 8 × 30 mm col 13 analgesic mix faster 1: Scan ES+ TIC 0.85 100-1.90e8 0.65 0.57 0.50 8-0.31 0.12 0.78 my 0 Time 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90

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🧱 Chromatogram - [13 analgesic mix faster] File Edit Display Process Window Tools Help - 8 × 😹 🖪 📾 🖻 🖻 🦀 🔛 🕰 🚺 🔵 A 🖷 🗗 🗘 Q⁺ Q^{*} TIC 🛊 🔺 💥 30 mm col 13 analgesic mix faster 1: Scan ES+ 0.85 TIC × 100-🗔 Integrate chromatogram 1.90e8 Noise OK Peak-to-peak amplitude g Cancel 0.65 Automatic noise measurement Copy Enable smoothing Smooth.. Paste ApexTrack Peak Integration Peak detect. Threshold.. 57 % 0.31 0.12 0.78 m 0 Time 0.60 0.10 0.30 0.40 0.50 0.70 0.80 0.90 0.20 Retention time window : 0.1032 0.1559 0.2591



Chromatogram - [13 analgesic mix faster] _ @ X 🜉 File Edit Display Process Window Tools Help ▲ 区 酸 動 助 個 L ▲ L ● A 書 話 Ø Q* Q* Q* TC ◆ ◆ X 30 mm col 13 analgesic mix faster 1: Scan ES+ TIC 0.85 100-🔜 Integrate chromatogram 1.90e8 Noise OK Peak-to-peak amplitude 552701.00 Cancel 0.65 Smooth chromatogram Window size (scans) ± 2 OK Number of smooths 1 Cancel Smoothing method Mean 🔘 Savitzky Golay 8 0.31 0.12 0.78 m him 0 Time 0.20 0.80 0.10 0.30 0.40 0.50 0.70 0.90 0.60



🧱 Chromatogram - [13 analgesic mix faster] _ 7 > File Edit Display Process Window Tools Help - 8 × 🖆 🗛 🗃 🛍 🛍 🛍 🔐 🔐 🖉 🏈 A 🖷 🛱 🖑 Q* Q* Q* 💜 💷 🚸 🕺 30 mm col 13 analgesic mix faster 1: Scan ES+ 0.85 TIC × 100-Integrate chromatogram 1.90e8 Noise OK Peak-to-peak amplitude 2552701 Cancel 0.65 Automatic noise measurement Сору Smooth. Enable smoothing Paste ApexTrack Peak Integration Peak detect. Threshold. 0.57 0.50 X 8 Relative height 1.50 OK Absolute height 10.00 Cancel Relative area 10.00 Absolute area 0.00 0.12 0.78 mym Time 0.10 0.20 0.30 0.40 0.50 0.70 0.80 0.60 0.90

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🗱 Chromatogram - [13 analgesic mix faster] _ 7 🗙 File Edit Display Process Window Tools Help - 8 × 🖆 🗛 👦 🛍 🛍 🛍 🔐 🕰 🔛 🍳 A 🖷 👘 🖑 Q* Q* Q* TIC 🍁 🚸 💥 30 mm col 13 analgesic mix faster 1: Scan ES+ TIC 0.85 × 100-🛄 Integrate chromatogram 1.90e8 Noise OK Peak-to-peak amplitude 2552701 Cancel 0.65 Automatic noise measurement Copy Smooth. Enable smoothing Paste Peak detect. ApexTrack Peak Integration Threshold... 0.57 0.50 8 0.31 0.12 0.78 m - Time 0.20 0.30 0.40 0.50 0.60 0.70 0.90 0.10 0.80

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ApexTrack Peak Integration

- Major Advantages
 - Peak apices detected by curvature
 - Apex detection independent of baseline determination
 - Reliable shoulder detection
 - Adjustable baseline criterion



Curvature of a Peak





Curvature of a Peak





Apex has high curvature. Inflection points have no curvature. Upslope/downslope has high curvature. Baseline has no curvature.

Curvature of a Peak





The Complete Second Derivative



Apex Detection



- Computation of second derivative
 - Automatic Determination of Peak Width
 - Automatic Determination of Apex Detection Threshold
- Detection of peak apices
 - Identification of Inflection points

Two Peaks

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Zoom in on Baseline

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Second Derivative





Zoom in on Second Derivative





AutoWidth





AutoThreshold





Apex Detection





Identify Inflection Points





Mark up Chromatogram



Apices and Inflection Points

Integrate chrom	atogram 🔀
Peak-to-peak amplitu	Ide 2552701 OK Cancel
Smooth	Enable smoothing Paste
Peak detect	ApexTrack Peak Integration
Threshold.	
ApexTrack Peak Detection Parameters	Response Threshold
Deal de Deal Pasalina Naisa 1400000	Absolute height
Peak-to-Peak baseline Noise 1450066	Relative area 2.00
	Absolute area 0.00
Baseline Start Threshold% 0.00	
Baseline End Threshold% 0.50	Set to 0.0 to see all detected peaks
Detect Shoulders	1
OK Cancel	Set to 100% to see inflection points

Baseline Determination

- Connect inflection points
 - Inflection points define initial baseline!
 - Final baselines satisfies % slope criterion.
- Form cluster baseline
 - From adjacent peaks
- Identify shoulders
- Compute Height, Area, Retention Time

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ApexTrack Peak Integration




Chromatogram - [13 analgesic mix faster] F 🐖 File Edit Display Process Window Tools Help - 8 × ▲ 区 酸 動 助 個 L ▲ L ● A = 話 Ø Q Q Q W T + ◆ X 30 mm col 13 analgesic mix faster 1: Scan ES+ TIC 0.85 × 100-Integrate chromatogram 1.90e8 Noise OK Cancel 0.65 Automatic noise measurement Copy Enable smoothing Smooth. Paste ApexTrack Peak Integration Peak detect... Threshold... 0.57 0.50 X 8-Automatic Peak-to-Peak Baseline Noise 1450066 Automatic Peak Width at 5% Height (Mins) 0.034 Baseline Start Threshold% 0.00 Baseline End Threshold% 0.50 Detect Shoulders 0.78 mm OK Cancel - Time 0.20 0.30 0.70 0.80 0.10 0.40 0.50 0.60 0.90

_ 7 × Chromatogram - [13 analgesic mix faster] File Edit Display Process Window Tools Help - 8 × 🖆 🗛 👦 🛍 🛍 🛍 🔐 🗛 🔛 🌒 A 署 👫 🖑 Q* Q* Q* 110 🍁 🔅 💥 30 mm col 13 analgesic mix faster 1: Scan ES+ TIC 0.85 100-Integrate chromatogram 1.90e8 Noise OK Cancel 0.65 Automatic noise measurement Copy Enable smoothing Smooth.. Paste ApexTrack Peak Integration Peak detect... Threshold.. 0.57 0.50 8 0.31 0.12 0.78 my 0 Time 0.40 0.50 0.60 0.10 0.80 0.90 0.20 0.30 0.70

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ApexTrack Peak Integration Summary

- Peak detection separate from baseline determination
- Robust shoulder detection
- Automatic parameter determination
- Rapid method development





Editing Detected Peaks

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Editing Detected Peaks



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Editing Detected Peaks



Peak Annotation

Chromatogram Peak Annotation	
Annotation Type Peak Top Time Peak Top Scan Peak Purity Decimal Places Scan Base Peak Mass	Annotation Threshold
Decimal Places 0 Peak Response Area Decimal Places 0 Peak Response Height Symmetry	BioLynx Component Label Digest Label Scan Set Mass Decimal Places OK Cancel



Peak Annotation

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Signal to Noise





Signal to Noise

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Signal to Noise

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Signal to Noise

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Spectrum



The Spectrum window is accessed from the MassLynx window by selecting spectrum from the sample list menu bar



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Displaying Spectra

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- Setting magnified ranges
- Peak annotation
 - Changing the number of decimal places for mass labeling
 - Labeling peaks relative to a reference peak
 - Setting the threshold for annotation
- Adding text to a spectrum
- Displaying a list spectrum



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Spectrum Annotation



· A B B B L 0 ● A B B 0	Q* Q~ Q* # ♦ ₩ \$			
nm col analgesic mix faster 150 (0.504) 152				1: Scan ES+ 7.55e
	Spectrum Peak Anno	otation		
	Decimal Places Mass Mass Error Component Label Intensity Intensity Error Annotation Threshold % Full Scale Intensity Intensity	Delta Mass Delta Mass I Digest Fragment La Transition State	0.00 Series abel	
110 111 130 135 145 174 183 199 201 222	Level	Нigh • ОК 09 327 341 357 34	Cancel	445 478479 494 m

Spectrum Annotation

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Spectrum Annotation

Spectrum Peak Anno	otation	X
Annotation Type		
Decimal Places	0	
Mass		
Mass Error	2 3 s Label Series	
Component Label	agment Label	
Intensity	Transition State	
Intensity Error		
Annotation Threshold		
% Full Scale	0.0	
OIntensity	0	
Level	High	

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Spectrum Annotation





Spectrum Annotation

1			
i			
Digest Fragment Label			
ncel			

Spectrum Annotation



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Waters[™] Spectrum Annotation ΠX Spectrum - [13 analgesic mix faster] File Edit Display Process Tools Window Help - 8 × 30 mm col 13 analgesic mix faster 150 (0.504) 1: Scan ES+ Spectrum Peak Annotation X 7.55e7 152.2_ 100 -Annotation Type -0.0 **Decimal Places** 1 × Mass V Delta Mass 152.20 Mass Error V Ion Series Label Series.. Component Label Digest Fragment Label Intensity Transition State Intensity Error Annotation Threshold 1.0 % Full Scale 0 O Intensity 8-Level ~ High OK Cancel 110.1 -42.1 153.4 135.3 -16.9 -7.0 247.0 94.8 266.2 288.1 309.1 327.0 14.0 135.9 156.9 174.8 341.1 204.9 212.9 402.8 411.0 250.6 258.8 436.0 292.7 283.8 283.8 478.5 494. , 326.3 341.9 326.3 341.9 500 183.0 199.0 221.9 30.8 46.8 69.7 0-100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460

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Spectrum Annotation



Adding Text to a Spectrum



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Adding Text to a Spectrum



Adding Text to a Spectrum



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Processing Spectra



- Combined spectra
- Combined spectra with background subtraction






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Spectrum - [13 analgesic mix faster] File Edit Display Process Tools Window Help 5 ▲ 🔉 🐲 🖻 🛍 📖 💷 🔵 A 📲 🖪 Ö Q⁺ Q⁻ Qኛ # Ê ۵ 🔹 30 mm col 13 analgesic mix faster 192 (0.645) Cm (190:194-(175:184+202:214)) 1: Scan ES+ 1.01e8 100-* 177 183 224 236 252 271 277 327 331 359 387 415 421 459 481 499 m/z 137 116 0 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500

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Chapter Seven

TargetLynx™

Quantitation



- Determines the concentration of specific analytes within a sample
- Can be done on data acquired through a variety of Acquisition Modes:
 - Multiple Reaction Monitoring (MRM)
 - Single Ion Recording (SIR)
 - Full Scan Acquisition

How Do We Quantitate?



- In addition to unknown samples, a set of standards is also run to form a calibration curve.
- MassLynx analyzes the response of unknown samples and compares their response to that indicated by the calibration curve, then calculates the concentrations of the unknowns.

Calibration Curve



Compound name: Parent Correlation coefficient: r = 0.998763; r*2 = 0.997527 Calibration curve: 0.555469 * x + -0.0034825 Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area) Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



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More On How Do We Quantitate?

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- Steps in Creation of a Calibration Curve for Quantitation:
 - In each chromatogram, determine the location of the peak relating to a specific compound
 - Integrate peaks in chromatograms
 - Calculate response factor for the located peak
 - Create a calibration curve for that compound

Quantification Example



- Set of analyses on samples using an MS method that has 2 MRM channels
 - Alprazolam (309.2 > 281)
 - Alprozolam-D5 (314.2 > 286.1)

Internal Standards



- Used to account for experimental drift
- Can be added at various points in the analysis
 - In the original sample
 - Before injection by the LC
- Response of analyte in a sample is:

(Peak Area of Analyte) (Peak Area of I.S.) / (Conc of I.S.)

Quantification Steps



- Enter sample types & concentrations into sample list
- Determine correct integration parameters for chromatogram peaks
- Create Quantification Method
- Process samples
- Check results adjust if needed
- Print out results save results in report File

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Set up Sample List

77 M	assLynx - drugmixture - alp	orazo	lam_quantify.SPL							
- <u>Fie</u>	• D B B Ø >		Shortcut	ueue <u> </u> Sta	atus					
					Queue I	s Empty				
뉟	TargetLynx @ Spectrum Chromatogram Map Edit → Samples →									
ne			File Name	File Text	MS File	Inlet File	Vial	Inject Volume	Sample Type	Conc A
I.I.		1	171208_Alpraz_QC_2096		Alprazolam	Alprazolam_4min	2:4	20.000	Standard	0.01
lst	Edit Method	2	171208_Alpraz_QC_2097		Alprazolam	Alprazolam_4min	2:5	20.000	Standard	0.05
F	Edit Hethod	3	171208_Alpraz_QC_2098		Alprazolam	Alprazolam_4min	2:6	20.000	Standard	0.1
ols		4	171208_Alpraz_QC_2099		Alprazolam	Alprazolam_4min	2:7	20.000	Standard	0.5
ĕ		5	171208_Alpraz_QC_2100		Alprazolam	Alprazolam_4min	2:8	20.000	Standard	1
	Process Samples	6	171208_Alpraz_QC_2101		Alprazolam	Alprazolam_4min	2:9	20.000	Standard	5
ize	0	7	171208_Alpraz_QC_2102		Alprazolam	Alprazolam_4min	2:10	20.000	Standard	10
OpenLynx QuanOptim	View Results TrendPlot									
TargetLynx	QCMonitor Email									

Set up Sample List



- Standard Sample list plus two additional categories:
 - Sample Type
 - Concentration A (B, C, D....)

	File Name	File Text	MS File	Inlet File	Vial	Inject Volume	Sample Type	Conc A
1	171208_Alpraz_QC_2096	~	Alprazolam	Alprazolam_4min	2:4	20.00	Standard	0.01
2	171208_Alpraz_QC_2097	2	Alprazolam	Alprazolam_4min	2:5	20.00	Standard	0.05
3	171208_Alpraz_QC_2098		Alprazolam	Alprazolam_4min	2:6	20.00	Standard	0.1
4	171208_Alpraz_QC_2099		Alprazolam	Alprazolam_4min	2:7	20.00	Standard	0.5
5	171208_Alpraz_QC_2100		Alprazolam	Alprazolam_4min	2:8	20.00	Standard	1
6	171208_Alpraz_QC_2101		Alprazolam	Alprazolam_4min	2:9	20.00	Standard	5
7	171208_Alpraz_QC_2102		Alprazolam	Alprazolam_4min	2:10	20.00	Standard	10

Set up Sample List – Load Format

Queue Is Empty Samples -Iram Map Edit-File Text MS File Sample Type Conc A Add I Standard 0.01 Alprazolam Insert Alprazolam I Standard 0.05 Delete Alprazolam I Standard 0.1 Fill ▶ I Standard Alprazolam 0.5 Clear Alprazolam ▶ I Standard 1 Column ▶ I Standard 5 Alprazolam Customize... Alprazolam Format Sort Load... Save... Number of Samples... Number of Injections... Load Sample List Format AutoSampler Bed Layout... OK C:\MassLynx\ MetaboLynx Acg Format ~ Cancel MetaboLynx Molecule Demo MetaboLynx MS/MS Demo Browse Metabonomics ProfileLynx ProfileLynxCalibration ProteinLynx.FMT proteinlynx_msms.fmt quantify.fmt Description Quantify sample list format

Review of Sample Types

- Blank Ensures that system is clean and/or shows endogenous material in sample.
- Solvent Similar to Blank sample type. This setting is used with the QC monitor feature.
- Standard Sample of a known concentration, used to form calibration curve.
- Analyte Sample of unknown concentration.
- QC Quality Control Known concentrations, used to test the validity and accuracy of the calibration curve.

Specify Sample Types and Concentrations

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 Pull Down menu within the sample list. Specify whether the sample is a Blank, Standard, Analyte, Solvent or QC.

Sample Type				
Analyte	*			
Analyte				
Blank				
Solvent				
QC				
Standard				
Recovery				
Donor				
Receptor				

• •
r

The known concentrations of Standards or QC's must be entered into this column.

Reference Samples

	Queue Is Empty								
Sp	ectrum Chromato	gram Ma	p Edit -	Samples -					
	File Name	File Text	MS File	Inlet File	Vial	Inject Volume	Sample Type	Conc A	Quan Referen
1	171208_Alpraz_QC_2096		Alprazolam	Alprazolam_4min	2:4	20.000	Standard	0.01	
2	171208_Alpraz_QC_2097		Alprazolam	Alprazolam_4min	2:5	20.000	Standard	0.05	
3	171208_Alpraz_QC_2098		Alprazolam	Alprazolam_4min	2:6	20.000	Standard	0.1	
4	171208_Alpraz_QC_2099		Alprazolam	Alprazolam_4min	2:7	20.000	Standard	0.5	
5	171208_Alpraz_QC_2100		Alprazolam	Alprazolam_4min	2:8	20.000	Standard	1	×
6	171208_Alpraz_QC_2101		Alprazolam	Alprazolam_4min	2:9	20.000	Standard	5	\sim
7	171208_Alpraz_QC_2102		Alprazolam	Alprazolam_4min	2:10	20.000	Standard	10	

Determining Integration Parameters

Instrument

Tools

QuanOptimize

TargetLynx OpenLynx

Quanpedia

QCMonitor Email

🍸 MassLynx - drugmixture - alprazolam_quantify.SPL File View Run Help 🖉 Shortcut 🗟 Queue 💁 Status 🥔 🔹 🗋 🔂 4 **Queue Is Empty** Spectrum Chromatogram Map Edit - Samples -TargetLynx 🕖 File Name File Text MS File Inlet File Vial Conc A Inject Volume Sample Type 2:4 11 171208_Alpraz_QC_2096 Alprazolam Alprazolam_4min 20.000 Standard 0.01 2 171208_Alpraz_QC_2097 2:5 0.05 Alprazolam Alprazolam_4min 20.000 Standard Edit Method 3 171208_Alpraz_QC_2098 20.000 Standard 0.1 Alprazolam Alprazolam_4min 2:6 4 171208_Alpraz_QC_2099 2:7 20.000 Standard 0.5 Alprazolam Alprazolam_4min (\mathcal{D}) 5 171208_Alpraz_QC_2100 2:8 20.000 Standard 1 Alprazolam Alprazolam_4min Process Samples 171208_Alpraz_QC_2101 2:9 20.000 Standard 5 6 Alprazolam Alprazolam_4min 80 7 171208_Alpraz_QC_2102 Alprazolam Alprazolam_4min 2:10 20.000 Standard 10 View Results TrendPlot 8

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Peak Integration - Display All Traces

Mass Chro	omatogram	\mathbf{X}
File: 171	ОК	
Ch1,Ch2	Cancel	
Function:	MRM of 2 Channels ES+	File
Channels:	1: 309.2 > 281 (Alprazolam) 2: 314.2 > 286.1 (AlprazolamD5)	Add trace Replace trace
		aew window



Peak Integration - Display All Traces



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Setup Peak Integration - Noise



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Setup Peak Integration-Smoothing



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Setup Peak Integration – Peak Detect

Peak Detection with Apex Integration



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Response Threshold

Integrate chromatogram			
Noise Peak-to-peak amplitude	OK Cancel		
Smooth	Copy		
Peak detect ApexTrack Peak Inter	gration	_14	
	Response Thresh	old	<u> </u>
	Relative height	1.50	ОК
	Absolute height	0.00	Cancel
	Relative area	2.00	
	Absolute area	0.00	

Setup Peak Integration Parameters



- Click **OK**, the peak of interest will be integrated.
- Review the integration is it acceptable? If not, repeat the integration with different parameters (noise, peak detect, thresholding) until satisfactory results are obtained.
- Once an acceptable integration is attained, you may want to test it on a low range standard and a high range standard to insure that parameters are adequate for the full range of response.

Review Peak Integration



 This is an example of a well integrated peak (left) and a poorly integrated peak (right).





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Build Quantitation Method

<u>File View Run Help</u>	
12 Unititied - LargerLynx Method Editor	
File Edit Update View Compound Help	
Compound List 🔒 🍇 🕍	
User Defined Properties Value	^
Compound Name	
F	
Acquisition Function Number 0	
Use absolute mass window (PBM) 0.0000	
For Four Mathematica For Four Fou	
Locate Peak Using	
Locate Peak Selection	=
Predicted Retention Time 0.0000	
Predicted Relative Retention Time 0.0000	
O Retention Time Window (mins) ± 0.0000	
Relative Retention Time Reference	
- Drococc Complex	
Process Samples Response Uses	
Response Type	
N Totals Group	
Totals Include	
Calibration Reference Compound	
Q View Results	
Concentration of Standard 0.0000	
Polynomial Type	
Propagate Calibration Parameters?	
× IrendPlot Smoothing Enabled? MNO	
Ready	NUM

C:\MassLynx\dru	gmixture.PRO\MethDB\setup.mdb - TargetLynx Metho	od Editor	
File Edit Update V	iew Compound Help		
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Compound List	S 2 2 4 4 4 4 5 % *		
1: Alprazolam	User Defined Properties	Value	
2: AlprazolamD5	Compound Name	Alprazolam	
	Acquisition Function Number	1	
	Quantification Trace	309.2 > 281	
	Use absolute mass window?	✓ YES	
	Locate Peak Using	Retention Time	
	Locate Peak Selection	Nearest	
	Predicted Retention Time	1.6200	
	Retention Time Window (mins) ±	0.0300	
	Relative Retention Time Reference	None	
	Response Uses	Area	
	Response Type	Internal (relative - use internal standards)	
	Totals Group		
	Calibration Reference Compound	1: Alprazolam	
	Concentration Units		
	Concentration of Standard: Level	Conc A	
	Polynomial Type	Linear	
	Propagate Calibration Parameters?	× NO	
	Smoothing Enabled?	✓ YES	
	ESmooth Parameters		
	Apex Track Enabled?	✓ YES	
	Apex Track Parameters		
	<		>
eady			NUM

Build Quantitation Method

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Build Quantitation Method

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Quant Method Editor – Add Info on Compounds



Untitled - TargetLynx Method Editor File Edit Update View Compound Help 🗋 🚔 🚽 🧐 🚽 - 🍕 🛷 📰 📖 Compound List 船 🖧 🗛 🖳 🖬 🛠 🐁 User Defined Properties Value Compound Name Acquisition Function Number 0 Quantification Trace × NO Use absolute mass window? Select Parameters to View Locate Peak Using Locate Peak Selection Compound Name Predicted Retention Time CAS Number Retention Time Window (mins) ± Relative Retention Time Reference Compound Type ☑Acquisition Function Number ☑ Quantification Trace Response Uses ✓Use absolute mass window? Response Type Chromatogram mass window (Da) ✓Locate Peak Using **Totals Group** ☑Locate Peak Selection Calibration Reference Compound ✓Predicted Retention Time ☑Retention Time Window (mins) ± ☑Relative Retention Time Reference **Concentration Units** ☑Response Uses Concentration of Standard: Level ☑Response Type □Internal Standards Polynomial Type ✓Totals Group Calibration Reference Compound Propagate Calibration Parameters? ☑ Concentration Units Smoothing Enabled? ☑ Concentration of Standard: Level ESmooth Parameters Stock Concentration Factor □User RF Value Apex Track Enabled? Apex Track Parameters < Ready OK Cancel



- TargetLyn>	Method Editor		
Jpdate View	Compound Help		
 Quantitation I 	on Ctrl+Q		
Noise Range	Ctrl+E	6 % .	
Second Targe	tion Ctrl+2 perties		Value
Third Target I	on Ctrl+3 ame		New Compound
Fourth Target	Ion Ctrl+4		
. Common and No	unction Num	ber	0
Compound Na	Liso absoluto mass window	w0	VES
	Chromatogram mass window	tow (Da)	0.0200
	onionatogram mass wind		
	Locate Peak Using		Retention Time
	Locate Peak Selection		Nearest
	Predicted Retention Time		0.0000
	Retention Time Window (r	mins) ±	0.2000
	Relative Retention Time Re	elerence	None
	Response Uses		Area
	Response Type		External (absolute - no internal standards)
	Totals Group		

'Right Click & Drag' 🚽 Untitled - TargetLynx Method Editor File Edit Update View Compound Help 2 🗆 A 200 00 D. * * • 0.01 C_2096 Sm (Mn, 1x2) 1.61;25301 171208_Alpraz_ 314.2 > 286.1 (AlprazolamD5) 100-1.33e6 Compound Properties Value Area Compound Name AlprazolamD5 8 Acquisition Function Number 1 >314.2 > 286.1 Quantification Trace Use absolute mass window? ✓ YES Chromatogram mass window (Da) 0.0200 1.05 1.59 1.60 1.61 1.62 1 63 1 64 1.66 171208 Alpraz QC 2096 Sm (Mn, 1x2) MRM of 2 Channels 1.62 309.2 > 281 (Alprazolam) 100-Locate Peak Using **Retention Time** 142 7.85e3 Area Locate Peak Selection Nearest Predicted Retention Time 1.6100 0.0100 Retention Time Window (mins) ± % **Relative Retention Time Reference** None **Response Uses** Area 0 - Time Response Type External (absolute -1.59 1.60 1.61 1.62 1.63 1.64 1.65 1.66 **Totals Group**

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Quantify Trace

- A single decimal number (m/z) for mass chromatograms from SIR or Full Scan data
- Two single decimal numbers separated by a ">" for an MRM function (e.g. 609.2 > 195.1)
- TIC for total ion current chromatograms
- BPI for base peak intensity chromatograms
- An1, An2, An3, or An4 for analog data
- The wavelength for DAD data
- Ch1, Ch2, etc. for SIR data to use one quantify method with multiple SIR functions. Ch1 is the first mass in the list, Ch2 is the second etc

Enter Compound Properties (Internal Standard)

Untitled - TargetLynx Method Editor File Edit Update View Compound Help 🚔 🚽 🎒 🍫 🛷 + 💩 🛷 😽 🔲 📖 Compound List 2 1: AlprazolamD5 **Compound Properties** Value **Compound Name** AlprazolamD5 Acquisition Function Number 1 Quantification Trace 314.2 > 286.1 Use absolute mass window? YES Chromatogram mass window (Da) 1.0 Locate Peak Using Retention Time Locate Peak Selection Nearest Predicted Retention Time 1.6100 Retention Time Window (mins) ± 0.0100 Relative Retention Time Reference None **Response Uses** Area Response Type External (absolute - no internal standards) **Totals Group**

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Enter Calibration Parameters (Internal Standard)

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Enter Integration Properties

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File Edit Update View	Compound Help	
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Compound List		
1: AlprazolamD5	Integration Properties	Value
	Compound Name	AlprazolamD5
	Smoothing Enabled?	VES
	Smooth Parameters	
	Smoothing Method	Mean
	Smoothing Iterations	1
	Smoothing Width	2
	Apex Track Enabled?	VES
	Apex Track Parameters	
	Peak-to-Peak Baseline Noise	× 169.9400
	Peak Width at 50% Height	▶ 0.039
	Baseline Start Threshold %	0.00
	Baseline End Threshold %	0.50
	Detect Shoulder Peaks?	NO NO
	EThreshold Parameters	
	Threshold Relative Height	☑ 1.50
	Threshold Absolute Height	× 0
	Threshold Relative Area	2.00
	Threshold Absolute Area	⊠ 0
	Integration Window Extent	1.00
	Propagate Integration Parameters?	VES

Enter Integration Properties

Integrate chromatogram	
Noise	ОК
Peak-to-peak amplitude 438	Cancel
Automatic noise measurement	Copy
Smooth Enable smoothing	Paste
Peak detect ApexTrack Peak Integr	ration
Threshold	

Edit Update \	/iew Compound Help	
Undo	Ctrl+Z 🖡 🐝 🔲 🛄	
Cut	Ctrl+X 🛛 🔁 🖶 🖼 📽 🐐	
Сору	Ctrl+C on Properties	Value
Paste	Ctrl+V Jund Name	AlprazolamD5
Copy Integrat	on Ctrl+A	VES
	Smooth Parameters	
	Smoothing Method	Mean
	Smoothing Iterations	1
	Smoothing Width	2
	Apex Track Enabled?	VES
	Apex Track Parameters	
	Peak-to-Peak Baseline Noise	169.9400
	Peak Width at 50% Height	🗵 0.039
	Baseline Start Threshold %	0.00
	Baseline End Threshold %	0.50
	Detect Shoulder Peaks?	NO NO
	Threshold Parameters	
	Threshold Relative Height	1 .50
	Threshold Absolute Height	⊠ 0
	Threshold Relative Area	2.00
	Threshold Absolute Area	× 0

Enter Target Ion Parameters

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Compound List	\$ <u></u> ≈ Z				
1: AlprazolamD5	Target Ion Properties	Value			
	Compound Name	AlprazolamD5			
	Quantification Trace	314.2 > 286.1			
	Use Quan Ion in Response Calculation?	VES			
Target Ion RT Window (mins) ± 0.0000					
Target Ion Ratio Method Quan/Ta Calculate Ion Ratio Tolerance As Ratio					
	EView First Target Ion Parameters				
	Target Ion Trace				
	Use trace in response calculation?	× NO			
	Target Ion Ratio	0.0000			
	Target Ion Ratio Tolerance (%) ±	0.00			
	Target Ion Must Exist?	NO NO			
	Target Ion Must Pass Ratio?	× NO			
	View Second Target Ion Parameters				
	View Third Target Ion Parameters				
	View Fourth Target Ion Parameters				

Reference Spectrum



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File Edit Update Viev	v Compound Help	
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Compound List	% * Z & & u z % *	
1: AlprazolamD5	Reference Spectrum Properties	Value
	Compound Name	AlprazolamD5
	Propagate Spectrum Settings?	NO NO
	Forward Fit Threshold	⊠ 0
	Reverse Fit Threshold	⊠ 0
	Propagate Spectrum Settings? Forward Fit Threshold Reverse Fit Threshold	× NO × 0 × 0

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Enter Calculation Factors

🚾 Untitled - TargetLynx Method Editor				
File Edit Update Viev	v Compound Help			
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Compound List	£ * Z ~ C C E Z *			
1: AlprazolamD5	Calculation Factors	Value		
	Compound Name	AlprazolamD5		
	User Peak Factor	0.0000		

QC Monitor Page

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File Edit Update View	Compound Help		
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Compound List	🧏 😤 Z A 4 🗳 🖬 🖬 ¥ 🐴		
1: AlprazolamD5	QCMonitor Properties	Value	
	Compound Name	AlprazolamD5	_
		and Barter and an angle of the second s	
	Blank Settings		
	Check Blanks?	NO NO	
	Solvent Blank Settings		
	Check Solvent Blanks?	✓ YES	
	Propagate Solvent Blank Settings?	NO	
	Maximum Solvent Blank Absolute Response	☑ 0.010	
	Action On Error	Continue Batch	
	■Calibration Quality Settings		
	Check Calibration?	× NO	
	EQC Settings		-
	Check QCs?	VES YES	
	Propagate QC Settings?	× NO	_
	Maximum Allowed Concentration Deviation	⊠ 0.00	
	Allow Greater Concentration Deviation Below Concentration	0.0000	
	Flag Error if Ion Ratios Out of Tolerance	NO NO	
	Flag Error if Retention Time Out of Tolerance	✓ YES	
	Flag Error if Peak Asymmetry/Shape Out of Tolerance	NO NO	
	Flag Error if Signal/Noise Out of Tolerance	NO NO	
	Action On Error	Reinject Sample	
	Maximum Reinjections	1	
	Reinject Failure	Run Shutdown	_
	EStandard Settings		_
	Check Standards?	× NO	
	Analyte Settings		-
	Check Analytes?	× NO	
Ready		NUM	1



Sample Flagging Page

A Untitled - Targetly	vnx Method Editor		
File Edit Undate View	v Compound Help		
Compound List			
1: AlprazolamD5	Sample Flagging Properties	Value	
· · · · · · · · · · · · · · · · · · ·	Compound Name	AlprazolamD5	
	Calibration Curve Settings		
	Signal To Noise Parameters		
	Propagate Signal To Noise Parameters?	× NO	
	Signal-to-noise method	RMS	
	Noise calculation factor	3.00	
	Noise window start (min)	0.0000	
	Noise window end (min)	0.0000	
	Measure peak signal level from	Peak Baseline	
	Detection Limit Factor	0.0000	
	Quantitation Limit Factor	0.0000	
	Flag Minimum Signal/Noise Ratio	≥ 2.00	
	ERetention Time Settings		
	Propagate Retention Time Parameters?	× NO	
	Predicted RT (mins)	1.6100	
	Flag RT Tolerance?	× NO	
	Lower Retention Time Tolerance (%)	0.00	
	Upper Retention Time Tolerance (%)	0.00	
	Concentration Flag Parameters		
	Maximum Concentration Limit	⊠ 0.0000	
	Reporting Concentration Limit	⊠ 0.0000	

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Adding to the TargetLynx Method

- This entire process now needs to be repeated for the two other compounds in the example.
- Parameters that may differ between compounds:
 - Name
 - Transition (Quantify Trace)
 - Integration Parameters
 - Internal Reference (Selecting an Internal Standard if used)
 - Concentration of Standards
 - Retention Time
 - Time Window
 - Response Type in General Parameters Window
 - Polynomial Type in the General Parameters Window

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Add Compound to List

ixture.PRO\MethDB\Qmeth1.mdb - Targ	etLynx Method Editor
Compound Help	
· • • •	
Compound Properties	Value
Compound Name	New Compound
Acquisition Function Number	1
Quantification Trace	
Use absolute mass window?	VES VES
Chromatogram mass window (Da)	1.0000
Locate Peak Using	Retention Time
Locate Peak Selection	Nearest
Predicted Retention Time	1.6100
Retention Time Window (mins) ±	0.0100
Relative Retention Time Reference	None
Response Uses	Area
Response Type	External (absolute - no internal standa
Totals Group	
	ixture.PRO\MethDB\Qmeth1.mdb - Targ Compound Help Compound Properties Compound Properties Compound Name Acquisition Function Number Quantification Trace Use absolute mass window? Chromatogram mass window (Da) Locate Peak Using Locate Peak Selection Predicted Retention Time Retention Time Window (mins) ± Relative Retention Time Reference Response Uses Response Type Totals Group



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Enter Calibration Properties (Analyte)

Qmeth1.mdb - TargetLynx Method Editor File Edit Update View Compound Help 🚰 🔒 🎒 🎲 🛷 + 💁 🖑 🗞 | Compound List **8 % Z A 4 4 5 % %** 1: AlprazolamD5 Value **Calibration Properties** 2: Alprazolam **Compound Name** Alprazolam Calibration Reference Compound 2: Alprazolam Concentration Units ng/mL Concentration of Standard: Level Conc A Polynomial Type Linear **Calibration** Origin Exclude Weighting Function 1/X Propagate Calibration Parameters? × NO

Enter Integration Properties (Analyte)

C:\MassLynx\drugmixture.PRO\MethDB\Qmeth1.mdb - TargetLynx Method Edito File Edit Update View Compound Help 🗋 🚅 🔒 🤹 💠 - 逸 🧇 🐥 🔳 📖 Compound List £ ≈ Z A € 4 E % % 1: AlprazolamD5 Integration Properties Value 2: Alprazolam Compound Name Alprazolam ✓ YES Smoothing Enabled? Smooth Parameters Smoothing Method Mean Smoothing Iterations 1 Smoothing Width 2 ✓ YES Apex Track Enabled? Apex Track Parameters Peak-to-Peak Baseline Noise **169.9400** Peak Width at 50% Height ▶ 0.040 0.00 Baseline Start Threshold % Baseline End Threshold % 0.50 Detect Shoulder Peaks? × NO Threshold Parameters ▶ 1.50 Threshold Relative Height × 0 Threshold Absolute Height Threshold Relative Area 2.00 Threshold Absolute Area × 0 2.00 Integration Window Extent Propagate Integration Parameters? ✓ YES

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Reference Spectrum



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Processing Samples

- Repeat the previous steps for additional compounds that would be present in your method.
- Once the entire method is built, it is time to process the samples.
- Highlight the samples to quantitate. If the entire sample list is to be processed, click on the upper left box to activate the entire sample list.
- Click TargetLynx then click Process Samples.

Processing Samples with TargetLynx

MassLynx - drugmixture - alpi <u>F</u> ile <u>V</u> iew <u>R</u> un <u>H</u> elp	razolam_quantify.SPL	
	Spectrum Chromatogram	Statu C Map Text
TrendPlot	1 171208 Create TargetLynx Dataset 2 171208 C:\MassLynx\drugmixture.PF 3 171208 C.\MassLynx\drugmixture.PF 4 171208 Operations 5 171208 Update Method Times 6 171208 Update Ion Ratios 7 171208 Integrate Samples Ø Quantify Samples Blank Subtract Print Quantify Reports Export Results to LIMS	Quantify From Sample 1 To Sample 7 Method: Qmeth1 Curve: Training Printing Report Format File: Cir LIMS Export File:

TargetLynx Browser

- The TargetLynx Browser is used to create and view TargetLynx datasets, print results or export results to other applications.
- A dataset contains the extracted raw data, methods, calibrations and results associated with a processed set of samples. All required processing can be performed from the TargetLynx Browser.
- The TargetLynx Browser file is automatically displayed when a Sample List is processed from MassLynx.

Viewing Resul	S Targetl vnx - untitled *		Maters
9	File Edit View Display Processing Window Help		V VOICIS
	😹 🔲 🔨 🚼 🖾 🖓 📭 📲 🖓 - 🍫 🚽 - 4		
		Alprazolam	
Cummon or	× #Name Type Std Conc	RT Area IS Area Response Primar Conc %Dev	
Summar	1 1 171208_Alpraz Standard 0.010	1.62 141.832 25423.432 0.006 bb 0.011 13.3	
Window	2 2 171208_Alpraz Standard 0.050	1.62 616.029 24661.928 0.025 bb 0.047 -5.6	
	3 3 171208_Alpraz Standard 0.100	1.62 1299.074 24644.279 0.053 bb 0.098 -1.5	
	4 4 171206_Alpraz Standard 0.500 5 5 171208 Alpraz Standard 1.000	1.62 63/4.223 24616.266 0.259 bb 0.460 -4.1 1.62 12970.400 24678.539 0.526 bb 0.973 -2.7	
	6 6 171208_Alpraz Standard 5.000	1.62 64934.422 23928.697 2.714 bb 5.018 0.4	
	7 7 171208_Alpraz Standard 10.000	1.62 130528.805 24056.088 5.426 bb 10.033 0.3	
			1
	17 Chromatogram	Calibration: 08 Apr 2009 16:09:39	
	171208_Alpraz_QC_2096 Smooth(Mn,1x2) MRM 0.01	of 2 channels,ES+ Compound name, Aprazolam 309.2 > 281 Correlation coefficient: r = 0.999936, r^2 = 0.999871	
	1.51	3.715e+004 Calibration curve: 0.540893 * x + -0.00054696 Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)	
	1.00	Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None	
	%-	¥	
	1.34		
	0-1		
	171208_Alpraz_QC_2096 Smooth(Mn,1x2) MRN	of 2 channels,ES+	
	0.01 AlprazolamD5	314.2 ≥ 286.1 1.326e+006	
		3	
	1324851	¥ 4.00	
		8 2.00	
	0.50 1.00 1.50 2 00 2.50 3.00	3.50 0.0 2.0 4.0 6.0 8.0 10.0	
	Ready	Alprazolam NUM	
	Analvte	Calibration	
©2025 Waters Corporation	Chromatogram	Curve	349

Results Window and Shortcuts

			Slide Show	Browse Sample	Brow Comp	vse ound						
2	J Tar	get	Lynx - untitlet *	đ								
F	ile E	dit	View Display	rocessing. Win	ndow Help							
C	28	1	. 😾 🖾 📮 🖣	. 48 8 ₽	4:0 - 0 {	}0 -				0 E		
F							Alprazola	m				
	;						Πριαχοία					
×		#	Name	Туре	Std. Conc	RT	Area	IS Area	Response	Primar	ng/mL	%Dev
T	1	1	171208_Alpraz	Standard	0.010	1.62	141.832	25423.432	0.006	bb	0.011	13.3
	2	2	171208_Alpraz	Standard	0.050	1.62	616.029	24661.928	0.025	bb	0.047	-5.6
	3	3	171208_Alpraz	Standard	0.100	1.62	1299.074	24644.279	0.053	bb	0.098	-1.5
	4	4	171208_Alpraz	Standard	0.500	1.62	6374.225	24618.268	0.259	bb	0.480	-4.1
	5	5	171208_Alpraz	Standard	1.000	1.62	12970.400	24678.539	0.526	bb	0.973	-2.7
	6	6	171208_Alpraz	Standard	5.000	1.62	64934.422	23928.697	2.714	bb	5.018	0.4
	7	7	171208_Alpraz	Standard	10.000	1.62	130528.805	24056.088	5.426	bb	10_033	0.3



Std. Conc. = Concentration from Sample List

Summary Window

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Change Column Order

2	Targ	etl	ynx - untitled *						
F	ile Ed	it	View Display Pr	ocessing Win	dow H	Help			
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								Alprazo	blam
×		#	Name	Туре	Sto	d. Conc	R	T Are:	al IS Areal Response Prim
	1	1	171208_Alpraz	Standard		0.010	1.6	2 141.83	Show Chromatograms
	2	2	171208_Alpraz	Standard	4 6	0.050	1.6	2 616.02	Exclude
	3	3	171208_Alpraz	Standard	2	0.100	1.6	2 1299.07	List By Sample
	4	4	171208_Alpraz	Standard		0.500	1.6	2 6374.22	Hide Column
Column Order								2 12970.40	Edit Column Properties
Available Columns		~		olumn Order	1		к	2 64934.42	Change Column Order
Available Columns Available Co	lag ag J ise ual) d) ise lag ag J ise		Add Sa Remove Sa Add All IS Remove All Pr Ca Ca	olumn Order ample Entry ample Name ample Type d. Conc und Peak RT aak Area Area aak Response imary Det Flags alculated Conc anc. Deviation		Car Prope	IK	2 130528.8(1 130	Sort Ascending Sort Descending Edit Field Display Options Curve type: Linear. Origin: Exclude. Weight

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Edit Column Properties

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				Alprazola	m			
# Name	Type	Std. Conc	RT	Area	IS Area	Response	Primary FI	ng/mL %Dev
1 171208_Alpraz	Standard	0.010	1.62	141.832	25423.432	0.006	bb	Show Chromatograms
2 171208_Alpraz	Standard	0_050	1.62	616.029	24661.928	0.025	bb	Exclude
3 171208_Alpraz	Standard	0.100	1.62	1299.074	24644.279	0.053	bb	List By Sample
4 171208_Alpraz	Standard	0.500	1.62	6374.225	24618.268	0.259	bb	Uida Calump
5 171208_Alpraz	Standard	1.000	1.62	12970.400	24678.539	0.526	bb	Fide Column
6 171208_Alpraz	Standard	5.000	1.62	64934.422	23928.697	2.714	bb	Edit Column Properties.
7 171208 Alpraz	Standard	10.000	1.62	130528.805	24056.088	5.426	bb	Change Column Order.
Column Properties					nd name: A	Iprazolam		Edit Field
Column Properties	Value	1		ОК	nd name: A on coefficier on curve: 0.5	lprazolam nt: r = 0.999936 540893 * x + -0.1 rnal Std / Ref 1	, r^2 = 0.99987 _ 00054696	Edit Field Display Options
Column Properties Property Name	Value Calculated Co	onc		ОК	nd name: A on coefficier on curve: 0.5 se type: Inter	lprazolam nt: r = 0.999936 540893 * x + -0. rnal Std (Ref 1	, r^2 = 0.99987 00054696), Area * (IS Cd.,	Edit Field Display Options
Column Properties Property Name Visible	Value Calculated Co Yes	DNC		OK Cancel	nd name: A on coefficier on curve: 0.5 se type: Inter	lprazolam nt: r = 0.999936 540893 * x + -0. rnal Std (Ref 1	, r^2 = 0.99987 00054696), Area * (IS Cc.,	Edit: Field Display Options
Column Properties Property Name Visible Heading	Value Calculated Co Yes ng/mL	onc		OK Cancel	nd name: A on coefficier on curve: 0.6 se type: Inter	lprazolam nt: r = 0.999936 540893 * x + -0. rnal Std (Ref 1	, r^2 = 0.99987 00054696), Area * (IS Cc.,	Edit: Field Display Options
Column Properties Property Name Visible Heading Heading Alignment	Value Calculated Co Yes ng/mL Right	onc		OK Cancel	nd name: A on coefficiei on curve: 0.5 se type: Inter	lprazolam nt: r = 0.999936 540893 * x + -0. mal Std (Ref 1	, r^2 = 0.99987 00054696), Area * (IS Cc.,	Edit: Field Display Options
Column Properties Property Name Visible Heading Heading Alignment Width [inch(es)]	Value Calculated Co Yes ng/mL Right 0.62	onc		OK Cancel	nd name: A on coefficier on curve: 0.9 se type: Inter	lprazolam nt: r = 0.999936 540893 * x + -0. mal Std (Ref 1	, r^2 = 0.99987 00054696), Area * (IS Cc.,	Edit Field Display Options
Column Properties Property Name Visible Heading Heading Alignment Width [inch(es)] Alignment	Value Calculated Co Yes ng/mL Right 0.62 Right	onc		OK Cancel	nd name: A on coefficier on curve: 0.3 se type: Inter	lprazolam nt: r = 0.999936 540893 * x + -0. rnal Std (Ref 1	, r^2 = 0.99987 00054696), Area * (IS Cc.,	Edit Field Display Options
Column Properties Property Name Visible Heading Heading Alignment Width [inch(es)] Alignment Format Types Device 10	Value Calculated Co Yes ng/mL Right 0.62 Right Decimal Plac	onc es		OK Cancel	nd name: A on coefficier on curve: 0.3 se type: Inter	lprazolam nt. r = 0.999936 540893 * x + -0. rnal Std (Ref 1	, r*2 = 0.99987 00054696), Area * (IS Cc.,	Edit Field Display Options
Column Properties	Value Calculated Co Yes ng/mL Right 0.62 Right Decimal Plac 3	onc es		OK Cancel	nd name: A on coefficie on curve: 0.3 se type: Inter	lprazolam nt. r = 0.999936 540893 * x + -0. rnal Std (Ref 1	, r^2 = 0.99987 00054696), Area * (IS Cc.,	Edit Field Display Options

Overview Table

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X	TargetLynx - untitled											
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				10.30	Alprazol	am						
×	# Name	Туре	Std. Conc	RT	Area	IS Area	Response	Primary Fl	ng/mL	%Dev		
H	1 1 171208_Alpraz	Standard	0.010	1.62	141.832	25423.432	0.006	bb	0.011	13.3		
	2 2 171208_Alpraz	Standard	0.050	1.62	616.029	24661.928	0.025	bb	0.047	-5.6		
	3 3 171208_Alpraz	Standard	0_100	1.62	1299.074	24644.279	0.053	bb	0.098	-1.5		
	4 4 171208_Alpraz	Standard	0.500	1.62	6374.225	24618.268	0.259	bb	0.480	-4.1		
	5 5 171208_Alpraz	Standard	1.000	1.62	12970.400	24678.539	0.526	bb	0.973	-2.7		
	6 6 171208_Alpraz	Standard	5.000	1.62	64934.422	23928.697	2.714	bb	5.018	0.4		
	7 7 171208_Alpraz	Standard	10_000	1.62	130528.805	24056.088	5.426	bb	10.033	0.3		
×												
	171000 Alara 00 0000	AlprazolamD5	Alprazolam									
	171206_Alpraz_QC_2096	1.035	0.017									
	171200_Alpraz_QC_2037	1 003	0.098									
	171208 Alpraz_QC_2099	1 002	0.480									
	171208 Alpraz QC 2100	1.004	0.973									
	171208 Alpraz QC 2101	0.974	5.018									
	171208 Alpraz QC 2102	0.979	10.033									
C	🔨 Chromatogram					Calibration	: 09 Apr 20	09 09:05:0)2			
	171208_Alpraz_QC_2096 Smoot 0.01 100]	th(Mn,1x2)	MRM	of 2 channe 309.2 3.715	IIS,ES+ A CC 2 > 281 5e+004 = Re CC	ompound name: A orrelation coefficie alibration curve: 0, esponse type: Inte urve type: Linear, (Alprazolam ent: r = 0.999936 540893 * x + -0. ernal Std (Ref 1 Origin: Exclude,	i, r^2 = 0.9998 00054696), Area * (IS C Weighting: 1/x	71 :onc: / IS Area) ;, Axis trans: Nor	e	-	
	%- 1.34 0- 	Alprazolam;1. 2.3 50 2.00	62;141.83;7033 32 3. 2.50 3.00	29 3.50	- min - Min		2.0	× 4.0	6.0 8	.0	→ 	
Re	eady						🔀 Alp	razolam			NUM:	

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Overview Window Options

🈼 TargetLynx - untitled * File Edit View Display Processing Window Help 2 🔒 Next Sample 1. 13 😍 🖌 🍕 👌 -Previous Sample Alprazolam Next Compound # Name Response Primary FI. Std. Conc RT Area IS Area ng/mL %Dev Previous Compound 1 17120 141.832 25423.432 0.011 0.010 1.62 0.006 bb 13.3 1 2 2 17120 0.050 1.62 616.029 24661.928 0.025 bb 0.047 -5.6 Next Sample Group 3 3 17120 0.100 1.62 1299.074 24644.279 0.053 bb 0.098 -1.5 Previous Sample Group 4 17120 0.480 0.500 1.62 6374.225 24618.268 0.259 -4.1 4 bb Show Chromatograms 5 5 17120 1.000 1.62 12970.400 24678.539 0.526 bb 0.973 -2.7 5.018 6 6 17120 5.000 1.62 64934.422 23928.697 2.714 bb 0.4 Default 7 17120 7 10.000 1.62 X Options Slideshow Summary Chromatogram Spectrum Calibration Overview Colors and Fonts Failed Peaks Slideshow prazolam 171208_Alpraz_ Options.. 11 Decimal Places 171208_Alpraz_QC_2097 1.004 0.047 No. Decimal Places 3 ~ 171208_Alpraz_QC_2098 1.003 0.098 171208 Alpraz QC 2099 1.002 0.480 171208_Alpraz_QC_2100 1.004 0.973 171208 Alpraz QC 2101 0.974 5.018 0.979 10.033 171208 Alpraz QC 2102 🔨 Chromatogram 171208_Alpraz_QC_2096 Smooth(Mn,1x2) MRM of 2 channels.E 0.01 309.2 > 3.715e+ 1.51 100-1.55 OK Cancel Help 96 Alprazolam; 1.62; 141.83; 7033 10.0 1.34 0.0 2.32 3.29 ng/mL 2.0 0.50 1.00 1.50 2.00 2.50 3.00 3.50 0.0 4.0 6.0 8.0 10.0 Y X Alprazolam Set Display Options NUM

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Transpose Overview Table

~		AlprazolamD5	Alprazolam		
	171208_Alpraz_QC_2096	1.035	0.011		Transpose Table
	171208_Alpraz_QC_2097	1.004	0.047]	Display Analytes Only
	171208_Alpraz_QC_2098	1.003	0.098		
	171208_Alpraz_QC_2099	1.002	0.480	1	
	171208_Alpraz_QC_2100	1.004	0.973		
	171208_Alpraz_QC_2101	0.974	5.018	1	
	171208_Alpraz_QC_2102	0.979	10.033		
1				÷	

	171208_Alpr						
AlprazolamD5	1.035	1.004	1.003	1.002	1.004	0.974	0.979
Alprazolam	0.011	0.047	0.098	0.480	0.973	5.018	10.033

Chromatogram Window – Display Options



Chromatogram Window – View Options

Options Chromatogram Spectrum Calibration Overview Colors and Fonts Summary Style Annotation Graph Header Compound ~ ~ ✓Process Description ✓Peak Top Time Show Sample Data Peak Response Area Peak Response Area(Scientific) Fill Trace Fill Detected Peaks Peak Response Height v × Dook End Markera Dook Detection Floor Decimal Places: 2 Y Normalize Data To: Baseline at Zero Display Acquisition Range: × O Baseline (Percent Full Scale): 0.40 Min. Height: O Lowest Point Link Vertical Axes OK Cancel Help

Waters[™]

Kalibration: 09 Apr 2009 10:16:24 Compound name: Alprazolam Correlation coefficient: r = 0.999936, r^2 = 0.999871 Calibration curve: 0.540893 * x + -0.00054696 Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area) Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None 10.0-Residual 5.0-Next Compound Previous Compound 0.0-Default Display Range × × X Display Options... -5.0ng/mL Properties... 5.00 4.00-Response 3.00-2.00-1.00 0.00 ng/mL 9.5 0.5 1.5 4.5 6.5 1.0 4.0 5.5 6.0 7.0 7.5 8.5 0.0 2.0 2.5 3.0 3.5 5.0 8.0 9.0 10.0

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Calibration Window

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Calibration Window – View Properties

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ummary	Chromatogram	Spectrum	Calibration	Overview	Colors and Fonts	ND.	
Graphs	ia di la constante di la consta						
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Dis	play RF Calibratio	on By Points					
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Hig	hlight Calibration	Point Assoc	iated with the	Current Sa	mple		
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Hig	hlight Calibration	Point Assoc	iated with the	Current Sa	mple		
□Hig	hlight Calibration	Point Assoc	iated with the	Current Sa	mple		
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Hig	hlight Calibration	Point Assoc	iated with the	Current Sa	mple		
Hig	hlight Calibration	Point Assoc	iated with the	Current Sa	mple		
Hig	hlight Calibration	Point Assoc	iated with the	Current Sa	mple		
Spectrum Window – View Properties

 Options
 Summary Chromatogram Spectrum Calibration Overview Colors and Fonts

 Style
 Annotation

 Ø Header
 Decimal Places for PICS:

 Ø Show Reference
 Ø Graph Header

 Ø Graph Header
 OK

 OK
 Cancel

Summary Window Properties



Color and Fonts Window





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Manual Integration



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Manual Integration



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Manual Integration



Manual Integration



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Report Format

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Open	Ctrl+0	430 000 - 4	{ }0 -	X
Close				Alpra
Save	Ctrl+S			, upre
Save As		Std. Conc	RT	A
Refresh		0.010	1.62	141.8
Assessed Distance		0.050	1.62	616.0
Accept Dataset		0.100	1.62	1299.0
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More on TargetLynx Quantification

- Printing Reports (File, Print Report). Besides a full report, results from a set range of samples can be printed.
- Screen and Report Format. A customized format can be saved in a file for later use.
- The TargetLynx method used with a report can be changed using (Edit, Quantify Method)
- Editing of Calibration Curve (Edit, Calibration Curve) allows excluding of specific data points. ('Right Click' on a point in a Calibration Curve and select 'Exclude Point').
- Reprocessing samples after editing the TargetLynx method (Process, Calculate)

Saving Results



- Everything is in one file
- This file can be viewed and reports printed at a later date without reprocessing data
- This file will contain:
 - Compound and Sample Summaries
 - Calibration Curves
 - Chromatograms
 - Experimental Record for Each Analysis Run
 - Quantitation Method

Titles Are Initial Capitalization Style, Blue Font, 20 Point Size

- Bullets are Arial font, 18 point, and black
- This slide master is called "Title and Content"
- It does not have a subtitle

Titles Are Initial Capitalization Style, Blue Font, 20 Point Size

Subtitle: Font is Arial, 16 point and text color is gray

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Two Column Master Slide/Two Line Header Slide: Titles Are Initial Capitalization Style, Blue Font, 20 Point Size

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Chapter Eight

Xevo TQ-S micro Maintenance

System Maintenance



- What to use and not to use with MS detector
- Good practices
- Troubleshooting
- Source Maintenance
 - Cleaning the Sample Cone and Cone Gas Cone
 - Cleaning the Source and Ion Block
- Probe Maintenance
 - Replacing the ESI Probe Stainless Steel Capillary

What to use with MS detector

- List of compatible acids, bases and organic solvent
 - Acids: Formic, acetic, trifluoroacetic acid (caution)
 - Base: Ammonium hydroxide
 - Organic: Methanol, Acetonitrile, Propan-2-ol, Acetone
- Preferred/compatible buffer salts
 - Formates, Acetates, Carbonates
 - Keep below 10mM
- Ion pairing reagent can be used but risk prolonged ion suppression
 - TFA, DFA, HFIP can be used
 - Must no use sulfonate salts

What not to use with MS detector

- Must not use non-volatile salts
 - Phosphates, nitrates
- Must remove as much detergents as possible from sample
 - SDS, PEG, TWEEN, Triton etc
 - Will result in ion suppression and persistent peak in chromatogram
- Chlorinated solvents, non-volatile organics and those not listed as usable in previous slide
- Strong acids and bases
- MS cleaning solution
- Concentrated buffered mobile phase
 - With the exception of HILIC, consult Waters before using

Good practices with MS

- Use clean solvents and fresh mobile phases
 - Prepare daily
 - Wash bottle thoroughly
 - Discard mobile phase if appears cloudy or particulates seen floating
- Respect the MS max capillary voltages
 - Positive 3.5kV, Negative 2.5 kV
 - Always start low and go higher for better signal intensity
- Inject samples previously cleaned up or treated
 - Dilute and shoot will contaminate your MS faster than you would expect
 - Unfiltered samples will clog your LC system and column
 - Incompatible solvents will cause LC relevant issues
- Make use of timed events, diverting LC flow to waste in between analyte detection window

Factors Affecting Performance


Checking the Instrument Performance

- Why In order to insure optimal performance of your system.
- How By injecting a test compound to check the parameters of your API/MS system
- Types of crucial parameters

Mass position:	Mass Calibration
Resolution:	Quad.
Sensitivity:	Ion source

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Corrective Actions

- Poor sensitivity
 - Main Cause : Dirty (polluted) optics (source)
 - Solution : Source and optics cleaning
- Inaccurate mass position
 - Main cause : Inadequate operating mass range or bad choice of calibration ions
 - Solution : Mass scale calibration
 - Do not attempt calibration unless you know how or engineer recommended it

Cleaning the Source Components

- When there is a noticeable drop in signal intensity that is not due to the sample or LC components of the system, clean the sample cone and the cone gas cone.
- The source block incorporates an isolation valve that lets you service the sample cone without venting the system.
- If cleaning the sample cone and cone gas cone fails to increase signal sensitivity, also clean the ion guide and ion block.
- The illustrated maintenance procedure can be found in the Xevo TQ-S micro operator's guide.

Hints and tips for sample cone cleaning

- There is no special cleaning required for the cone.
- 50:50 MeOH:Water 10% formic sonicate for 30 minutes then sonicate
- Discard and rinse then fill with water, sonicate for a couple of minutes
- Discard and cover with methanol sonicate for a couple of minutes
- Blow the methanol off with clean air or nitrogen ensuring no solvent staining

NB – Later work has demonstrated that in some instances cleaning inside the cone is difficult using formic acid and 10% Nitric acid has been used as an alternative Its not uncommon to get a bias in the location of build-up



Replacing the Tool Free ESI Probe

- The stainless steel capillary should be replaced when:
 - There is a noticeable drop in signal intensity or
 - Lack of signal accompanied by an increase in backpressure, not related to the LC components of the system.
- The illustrated maintenance procedure can be found in the Xevo TQ-S micro operator's guide.

Xevo TQ-S micro: Troubleshooting

- Source Drawings
 - No signal in the Tune Page
 - Basic Troubleshooting: Fluidics and MS default parameters
 - Advanced Troubleshooting: The Diagnostic Page
- Miscellaneous Analytical Related Problems
 - Poor Sensitivity
 - Poor Resolution
 - Unstable Signal
- Miscellaneous Instrument Hardware Related Problems
 - Poor Vacuum
 - Heating Issues
 - Unstable Gas Flow and related issues (Nitrogen)

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Xevo TQ-S Micro Basic Troubleshooting Guide: No Ions



- Basic Control of the Fluidics
 - Check that the isolation valve is open on the source
 - Check all connections (from 1 to 8), check the UV flow cell
 - Disconnect at a specific location to see if there is a liquid coming out
 - Change any suspect parts of tubing or/and damaged nuts
 - Check to see if liquid is coming out at the tip of the ESI capillary

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Xevo TQ-S Micro Basic Troubleshooting Guide: No Ions



Xevo TQ-S Micro Basic Troubleshooting Guide: No Ions Fluidics Waters



Miscellaneous Analytical Related Problems

- Poor sensitivity
 - Dirty Source
 - Clean the Sample Cone and eventually the Ion Block
 - Leak located at the ESI/APCI connection (sample lost)
 Fix the leak
 - Resolution too high
- Poor Resolution (poor peak shape)
 - Resolution settings too low and/or ion energy too high
 - Dirty optics
 - Clean source ion guide and collision cell

Instrument Hardware Related Problems

- Poor Vacuum
 - Leak between the rough pump and the MS
 - Check the vacuum hose connections at the back of the MS and at the top of the pump
 - Leak located in the source area
 - Occurs most of the time when the source has been replaced after a cleaning procedure
 - o Dismantle the source and carefully check that all O-rings are well fitted
 - Leak at Ion Block Plug

Instrument Hardware Related Problems

- Poor Vacuum (cont')
 - Poor efficiency of the primary pump
 - Ballast pump for 1 hour, change the oil if necessary
 - Turbo speed too slow
 - Occurs when there is a leak located at the source or in between the MS and the rough pump

0

Always between 97 and 100%

Instrument Hardware Related Problems

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Unstable Gas Flow

- No gas flow or very low flow into the MS
 - $_{\odot}$ Leak located on the tubing between the gas delivering system and the MS nitrogen inlet
 - The gas regulators utilized in the Xevo TQ-S micro require at least 7 bars/100 psi to work correctly and accurately deliver stable gas flow rates. Check the pressure on the gas cylinder.
 - If you use a gas generator, check that the air compressor delivers the requested flow to the generator
- Excessive nitrogen consumption
 - o Check for leaks the entire gas line
 - o Check if the nebulization and desolvation gas inlets on the source have not been reversed

Summary

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- Routine maintenance is required to keep the instrument working properly and acquiring quality data.
- Frequency of source cleaning will be due to numerous factors, including mobile phase composition, sample type, and number of injections.
- Clean the source when proper resolution and signal intensity cannot be achieved for a known reference compound.
- Visit <u>https://support.waters.com/</u> for learning more about errors seen on instrument or MassLynx
- Read Overview and Maintenance Guide <u>https://www.waters.com/webassets/cms/support/docs/715004599v04.pdf</u> for more detailed information on the MS system
- Comprehensive guides <u>https://www.waters.com/nextgen/hk/en/education/primers.html</u>