



MxP® Quant 500 Kit

User Manual MxP® Quant 500 Kit – SCIEX Edition

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UM-MxP500-SCIEX-1
For Research Use Only. Not for use in diagnostic procedures.

MxP® Quant 500

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The information in this manual is subject to change without notice and should not be construed as a commitment by Biocrates® Life Sciences AG to assume responsibility for any errors that may appear. This manual is believed to be accurate for preparing the MxP® Quant 500 Kit and for using the Met/DQ™ Software. **The MxP® Quant 500 is for research use only and not for use in diagnostic procedures.** While every precaution has been taken in the preparation of this manual, Biocrates® Life Sciences AG shall not be liable for punitive, incidental, or consequential damage in connection with or arising from the use of this manual.

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Symbols

Symbol	Description
	LOT No.
	Product No.
	Ordering No.
	Expiration date
	Store at room temperature
	Store in freezer
	Store in refrigerator
	Note: pay attention to the user manual
	Manufacturer
	Not for reuse
	Attention
	Comment

Symbol	Description
!	Important information
	Acidic
	Irritant
	Highly flammable
	Toxic

1 About the Kit

MxP® Quant 500

The MxP® Quant 500 is based on a combination of several experimental steps. Therefore, **it is required to read this user manual in detail** before proceeding with the analysis of the kit. If you need support, please find the contact details on the last page of this manual. You also will find links to our [video tutorials](#) and [FAQ system](#).

The MxP® Quant 500 is available for the SCIEX 5500 series mass spectrometers coupled to a UHPLC system.

The Kit combines flow injection analysis (FIA) and liquid chromatography (LC) methods. The Kit can be used in a variety of application areas, such as biomarker discovery, disease phenotyping, clinical research, pharmaceutical R&D, nutritional or analysis of environmental effects. The assay quantifies the following compound classes:

- alkaloids
- amine oxides
- amino acids
- amino acid related
- bile acids
- biogenic amines
- carbohydrates and related
- carboxylic acids
- cresols
- fatty acids
- hormones and related
- indoles and derivatives
- nucleobases and related
- vitamins and cofactors
- acylcarnitines
- lysophosphatidylcholines
- phosphatidylcholines
- sphingomyelins
- ceramides
- dihydroceramides
- hexosylceramides
- dihexosylceramides
- trihexosylceramides
- cholesteryl esters
- diglycerides
- triglycerides

The data acquisition is performed using specific mass transitions (MRM pairs). For quantitation, calibration standards in seven concentration levels and stable isotope-labeled internal standards (ISTD)

are used. The filters of the Kit plate contain all internal standards. The analytical performance is validated using quality controls (QCs) at three concentration levels. The Biocrates® proprietary Met/DQ™ software is an integral part of the Kit and must be installed before starting with the kit preparation. Please refer to the Met/DQ™ manual “UM-Met/DQ-version-#.pdf” on the USB stick.

The MxP® Quant 500 was validated with human EDTA plasma . Both EDTA and heparin are suitable anticoagulants. Due to its biological similarity, human serum can also be used without impairing the analytical performance. For other biological matrices and species, please refer to our provided application notes (USB stick and <http://www.biocrates.com>). On [Biocrates' homepage](#), guidelines for the collection and preparation of biological samples are provided (“Sample Preparation Guidelines”).

Up to 78 samples can be analyzed with a full 96-well Kit depending on the number of quality controls (QCs), see info box below. The remaining wells are reserved for one blank, three zero samples, seven calibration standards, and three QCs. QC2 must be measured at least in replicates of five. The required laboratory equipment is listed in section 2: *Required Equipment and Materials (Not Supplied)*.

Important: To generate reproducible and longitudinal comparable data, do not change the acquisition method, quantitation method, or software version within one study.
For LC data quantitation use either Met/DQ™ or the Analyst® software.



Analyzing QC level 2 in replicates of five. This guarantees higher quality data when normalizing data across several plates (see Appendix “Data Normalization” in the Met/DQ user manual). For more information refer to the [EMEA guidelines for bioanalytical method validation](#) (European Medicines Agency, 2011).



If samples from a different matrix than plasma are analysed, use a QC of the corresponding matrix. Run this QC in replicates of five.

Note: if no plasma sample are used, Biocrates QCs may not be analysed in replicates.

Shipping and Storage Information

After receiving the MxP® Quant 500 Kit:

1. Store the MxP® Quant 500 plate and the vial box at -20 °C or -80 °C. The respective expiration date is shown on each item and on the side label of the kit box.
 2. Store all other items at room temperature.
 3. Keep the MxP® Quant 500 plate sealed in the plastic bag until use.
- ⓘ Biocrates® can only guarantee proper performance of the kit when used prior to the expiration date and stored under the recommended conditions.

The MxP® Quant 500 is shipped in one package, except for dry ice shipments. Keep the MxP® Quant 500 plate in the plastic bag sealed under nitrogen until use. Biocrates® can only guarantee a proper kit performance if the plate is stored unopened in its original plastic bag and away from light. Vial box and kit plate must be stored at -20 °C or -80 °C . All other items can be stored at room temperature. Please find the respective expiration dates on the individual items and on the side label of the kit box.



1.1 MxP® Quant 500 Contents

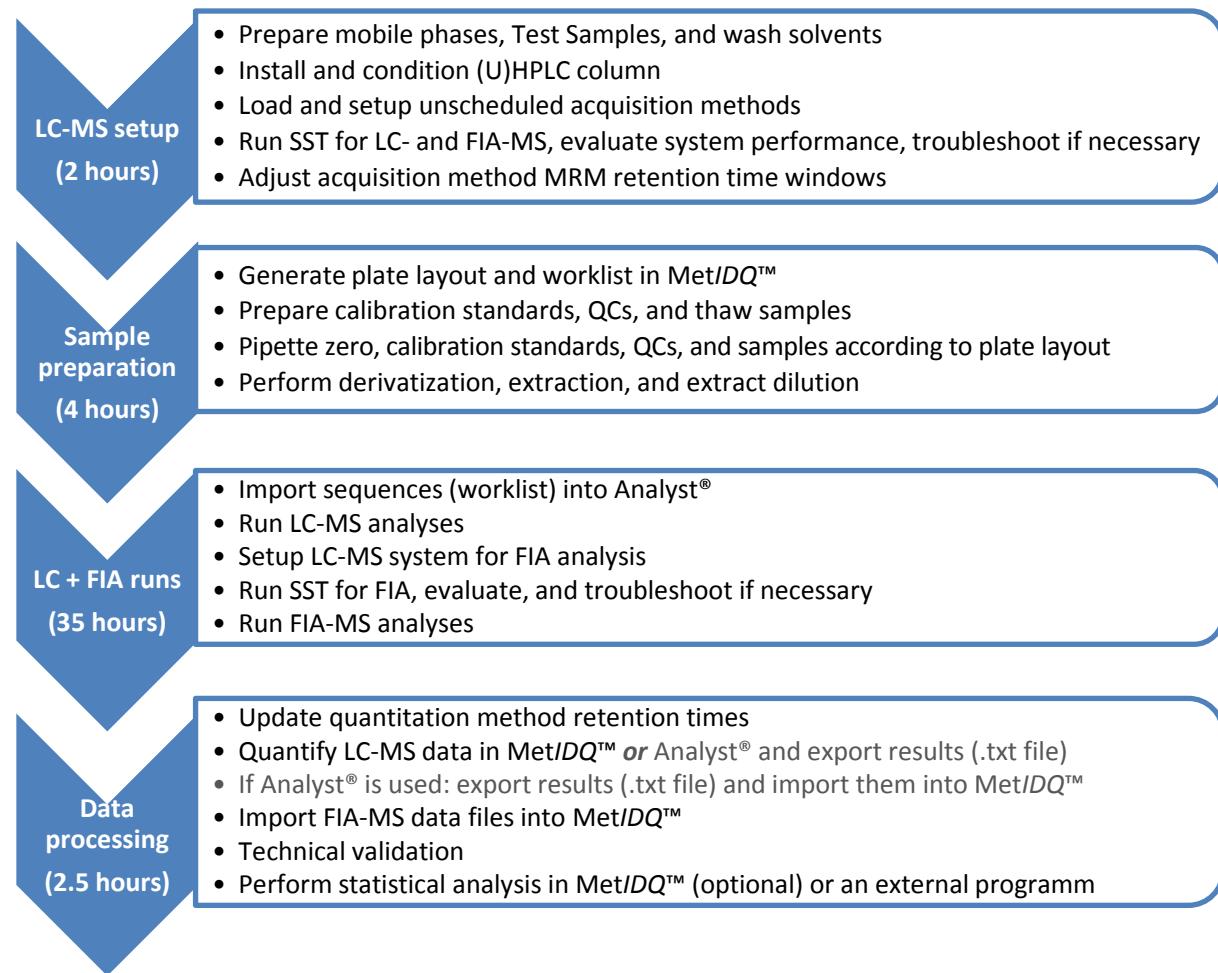
Kit Item	Description	Details
MxP® Quant 500 plate, 1 item	Plate stack consisting of a filter plate and a capture plate that are attached with tape	<i>The kit plate was sealed under nitrogen in a plastic bag. Do not open until starting kit preparation.</i>
96 deep well plates, 2 items	Empty plates	<i>Used to dilute the extracts after sample preparation.</i>
Silicone mats, 2 items	Silicone coverings for 96-well plates	<i>Used to seal the plates after preparation.</i>
FIA Mobile Phase Additive, 2 items (1 spare)	Sealed glass ampule	<i>Component for preparing FIA solvent.</i>
Tube for derivatization solution, 1 item	Empty plastic tube	<i>Used for preparing PITC solution.</i>
Test Sample LC, 1 vial	Biocrates® Test Sample for LC part (dried)	<i>Human plasma based sample for LC system suitability test; see section 6.</i>
Test Sample FIA, 1 vial	Biocrates® Test Sample for FIA part (dried)	<i>Human plasma based sample for FIA system suitability test; see section 6.</i>
Quant 500 QC, 3 vials	Biocrates® quality control Samples (lyophilized plasma), blue, yellow, green caps	<i>Spiked human plasma in different concentration levels.</i>
Quant 500 Cal, 7 vials	Biocrates® calibration standards (lyophilized), red caps	<i>Calibration standards used for LC-MS quantitation.</i>
USB stick		<i>Content described below.</i>
Met/DQ™ software and modules	Current version.	Provided with USB stick.
OracleXE (Express Edition) Database	64-bit version	Provided with USB stick.
User manual	UM-MxP500-SCIEX-#.pdf	Read carefully before using the kit. Provided with USB stick.

Kit Item	Description	Details
User manuals for Met <i>IDQ</i> ™ software and modules	UM-Met <i>IDQ</i> -version-#.pdf UM-StPk-#.pdf	Read carefully before using the kit. Provided with USB stick.
Application notes	Kit applications using different sample materials and species	Provided with USB stick
SOPs	Protocols for the analysis of different matrices	Provided with USB stick
Guidelines for sample collection	Guidelines for collecting plasma, serum and tissue samples	Provided with USB stick
Safety data sheets (SDS)	SDS for kit components.	Provided with USB stick

Instrument specific files on USB stick

Kit Item	Details			
Acquisition methods for Analyst®	LC	5500 series	UHPLC	MxP500L-LC1_5511.dam MxP500L-LC2_5511.dam
	FIA		FIA	MxP500F-FIA1_5501.dam MxP500F-FIA2_5501.dam
Quantitation methods for Analyst®	LC	5500 series	UHPLC	MxP500L-LC1_IntelliQuan_5511.qmf MxP500L-LC2_IntelliQuan_5511.qmf
Excel file	Calibration Standard Concentrations_LC1.xlsx			

1.2 Workflow at a Glance



Always check the LC-MS performance before you start with the Kit preparation. Note that the time designations are approximate.

2 Required Equipment and Materials (Not Supplied)

Instrumentation, laboratory equipment, chemicals, and solvents listed below are required to use the MxP® Quant 500 but are not provided with the MxP® Quant 500.

2.1 Mass Spectrometer and Laboratory Equipment

Material/Instrument	Specifications
Mass Spectrometer	<ul style="list-style-type: none">SCIEX 5500 series <p><u>Ion source:</u> TurboV™</p>
LC System	Binary UHPLC system with degasser unit and column oven <p><u>Injection volumes:</u> 5 – 20 µL</p>
Autosampler	A 96-well plate autosampler with temperature control (10 °C) and with an injector capable of piercing a silicone mat. For a flow through needle autosampler, a “sandwich injection” is required, refer to page 23.
Column Oven	Column oven, 50 °C
Column	Biocrates® MxP® Quant 500 UHPLC column (Biocrates® Part No.: 22005)
Tubing	Tubing for UHPLC with inner diameter of 130 µm / 0.005 in (color code red), e.g. Thermo Scientific™ Viper™ Capillary 0.13 x 350 mm (6040.2335)

Material/Instrument	Specifications	
Nitrogen evaporator or pressure manifold	Nitrogen evaporator for 96 well plates, vendors e.g. Techne, Porvair, VLM or Organomation	Positive Pressure Manifold for 96 well plates, e.g. Waters® Positive Pressure-96 Processor or Biotage® PRESSURE+ 96 Manifold
	Note: the evaporator or pressure manifold must be located in a fume hood. If a pressure manifold is used, a plate centrifuge is not required.	
Centrifuge	Must be able to centrifuge 96-well plates of 5 cm height at 500 x g	Not required when a pressure manifold is used
Nitrogen supply	Minimum pressure requirement of 4 bar	
Solvent bottles	From 50 to 1000 mL	
Balance	Accuracy < 1 mg	
Plate shaker	e.g. Eppendorf® ThermoMixer® or MixMate®	
Vortexer	Any model	
Pipettes	<ul style="list-style-type: none"> • Single channel: volume range from 10 µL to 1000 µL • Repeater: using 1.0, 2.5, and 10 mL tips, e.g. Eppendorf® Multipette® E3 (Ordering No. 4987000010 or 4987000371) • 8-channel: volume range from 50 µL to 1200 µL, e.g. Eppendorf® Xplorer® plus (Ordering No. 4861000821) 	

2.2 Chemicals and Solvents

Solvents/Chemicals	Specifications
Water	LC-MS grade or Milli-Q®
Ethanol, methanol, acetonitrile, water, and isopropanol	LC-MS grade
Formic acid	LC-MS grade (e.g. ThermoFisher Scientific 85171)
Phenylisothiocyanate (PITC)	sequencing grade (e.g. Sigma Aldrich 317861)
Pyridine	LC-MS grade (e.g. ThermoFisher Scientific 25104)
Ammonium acetate	LC-MS grade (e.g. Sigma-Aldrich 73594)
Phosphate buffered saline (PBS)	p.a. grade (e.g. Sigma Aldrich P4417)

2.3 Software

Software	Required version	Details
Analyst®	Version 1.5.2 or later	Used for data acquisition. Optional: used for data quantitation
Oracle® database	<u>Option 1 (recommended):</u> Full commercial version <u>Option 2:</u> Oracle XE, free of charge, provided on kit USB stick. No support and updates provided by Oracle®.	Required for MetIDQ™
Biocrates® MetIDQ™ Nitrogen	Provided by Biocrates®	Sample registration, kit validation, data exportation, and statistical analysis (see UM-MetIDQ-Nitrogen-#.pdf)



Define “English US” or “English UK” regional settings on the PC on which Analyst® and/or MetIDQ™ are installed. The “decimal symbol” must be set to “point” and the “digit grouping symbol” to “comma”. Go to **Windows Control Panel > Regional and Language > Formats > Additional Settings > Numbers**.

3 Safety Instructions

3.1 Safety Instructions for Personal Protection

The mobile phases (solvents) and most reagents are classified as “hazardous substances”. The sample preparation must be carried out in a fume hood in a laboratory or other location in full compliance with local guidelines. Due to the use of dangerous solvents (e.g. methanol, acetonitrile), the LC-MS system must be properly ventilated. The calibration standards are matrix-free. The QCs are human plasma based, tested to be free from known pathogens (hepatitis B and C, HIV 1 and 2, syphilis). However, materials should still be considered potentially infectious. For this reason, we recommend treating the QCs and your samples with an equal level of precaution. The kit must be processed by trained personnel, such as MTA, CTA, BTA, or higher.

3.2 Safety data sheets

Safety data sheets (SDS) are provided for kit components on the USB stick, located in the folder “MxP 500\Documents and Notes\SDS”. In addition, SDS are available on request. For contact details please refer to page 130.

3.3 Proper Disposal of Laboratory Waste

Proper disposal of laboratory waste requires that all waste is collected and separated according to chemical composition. Unused ampules should be opened and contents disposed as organic halogen-free solvent.

4 Instrumental Setup



It is required that the laboratory staff is familiar and experienced with the mass spectrometer and the operating software.

Acquisition Methods are provided on the USB stick for each MS platform.

<i>MS Instrument</i>	5500 series
<i>LC variant</i>	UHPLC
<i>LC methods</i>	MxP500L-LC1_5511.dam MxP500L-LC2_5511.dam
<i>FIA methods</i>	MxP500F-FIA1_5501.dam MxP500F-FIA2_5501.dam

Quantitation Methods are provided on the USB stick for each MS platform.

<i>MS Instrument</i>	5500 series
<i>LC variant</i>	UHPLC
<i>LC methods</i>	MxP500L-LC1_IntelliQuan_5511.qmf MxP500L-LC2_IntelliQuan_5511.qmf

4.1 Autosampler, Column Oven, and Pump Settings

Provided acquisition methods contain only MS parameters. Further required settings for autosampler, pump, and column oven must be added manually. Detailed are described in sections 4.1, 4.3, and 4.4.



For details, refer to sections 11.1 *LC Settings* and 11.2 *MS Settings*.

4.1.1 Autosampler

Tubing:

Tubing with an inner diameter of 130 µm / 0.005 in is required. For the LC part use tubing suitable for UHPLC (up to 1000 bar), e.g. Thermo Scientific™ Viper™ Capillary 0.13 x 350 mm (6040.2335).

LC part | between autosampler and column:

- Use tubing suitable for UHPLC. Inner diameter of 130 µm / 0.005 in.

LC part | between column and ion source:

- Use tubing suitable for HPLC or UHPLC. Inner diameter of 130 µm / 0.005 in.

FIA part | between autosampler and ion source:

- Use tubing suitable for HPLC or UHPLC. Inner diameter of 130 µm / 0.005 in.

LC injections – flow through needle systems (FTN):

For best sensitivity and chromatographic performance, a “sandwich injection” procedure is used. 5 µL per sample are injected together with 10 – 15 µL water, depending on the autosampler used.

The “sandwich injection” procedure consists of two steps. Water and sample are placed one after the other in the sample loop at the same time.

1. 10 – 15 µL water are loaded.
2. 5 µL sample are loaded.



Water is injected before the sample. On its way to the column, the organic solvent percentage of the sample is reduced by dilution with water. As a result, the chromatographic performance increases, which guarantees baseline separation of several metabolites, e.g. ADMA and SDMA.

Note: The “sandwich injection” procedure may only be required for flow through needle systems (FTN).

A detailed description of the “sandwich injection” procedure for different autosampler types can be found in Table 1. For loop based systems, refer to the next paragraph.

LC injections – loop based systems:

If a loop based injection system is used, install a 20 µL sample loop. Use a sample injection volume of 5 µL. Flush out the sample in the same direction as the sample was injected into the sample loop – the same flow direction through the sample loop is required for loading and injecting the sample.



Before the sample is injected into the sample loop, the system is flushed with solvent A (water, 0.2 % formic acid). While the sample is loaded and subsequently flushed with solvent A, it is mixed with water. As a result, the chromatographic performance increases, which guarantees a baseline separation of several metabolites, e.g. ADMA and SDMA.

FIA injections:

Standard sample injection used. Injection volume is 20 µL.

Table 1 “Sandwich injection” procedure and plate types:

Manufacturer	Rack Code	Plate type	“Sandwich injection” procedure
Agilent	2 Well Plates	96DeepNunc31mm	Section 11.1.4

Kit installation only: to adjust the settings of your autosampler for the MxP® Quant 500, use the 1 mL 96 deep well plate with a silicone mat that you received with the *Setup Box*.

- Check the needle penetration depth using the provided 1 mL 96 deep well plate. An appropriate position is approximately 1 mm above the bottom of the well.

Integrate rinsing steps to avoid cross contamination.

Wash solvents see 5.4 *Autosampler Wash Solvents*.

4.1.2 Column Oven

Temperature: 50 °C

4.1.3 Pump

UHPLC System

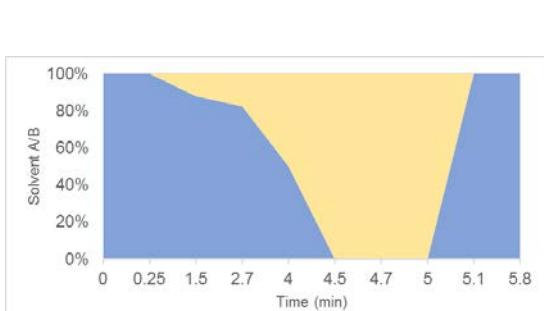
Solvents: Solvent A and Solvent B (see 5.2)

UHPLC gradient – LC1 part

Method: *MxP500-LC1_5511.dam*

Column: *Biocrates® UHPLC column*

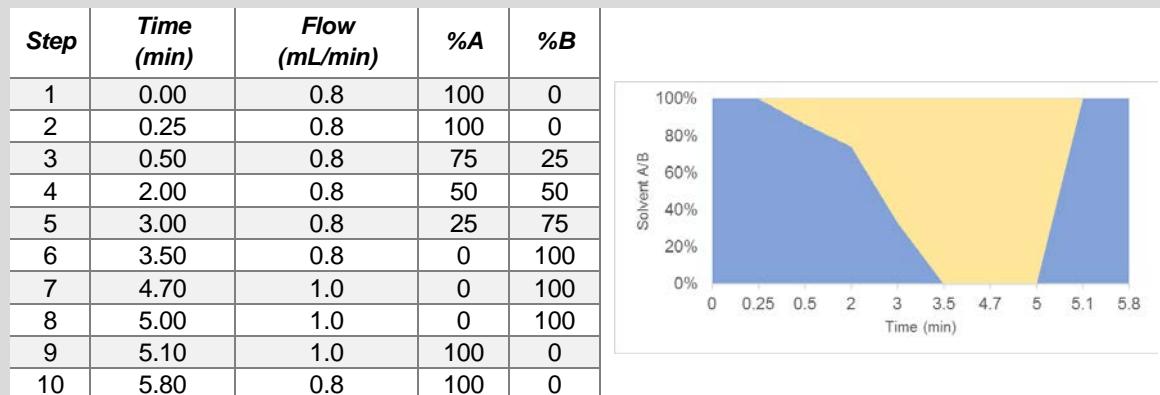
Step	Time (min)	Flow (mL/min)	%A	%B
1	0.00	0.8	100	0
2	0.25	0.8	100	0
3	1.50	0.8	88	12
4	2.70	0.8	82.5	17.5
5	4.00	0.8	50	50
6	4.50	0.8	0	100
7	4.70	1.0	0	100
8	5.00	1.0	0	100
9	5.10	1.0	100	0
10	5.80	0.8	100	0



UHPLC gradient – LC2 part

Method: MxP500-LC2_5511.dam

Column: Biocrates® UHPLC column



Gradient – FIA part

Methods: MxP500F-FIA1_5501.dam, MxP500F-FIA2_5501.dam

Step	Time (min)	Flow (mL/min)	%A	%B
1	0.0	0.03	0	100
2	1.6	0.03	0	100
3	2.4	0.20	0	100
4	2.8	0.20	0	100
5	3.0	0.03	0	100

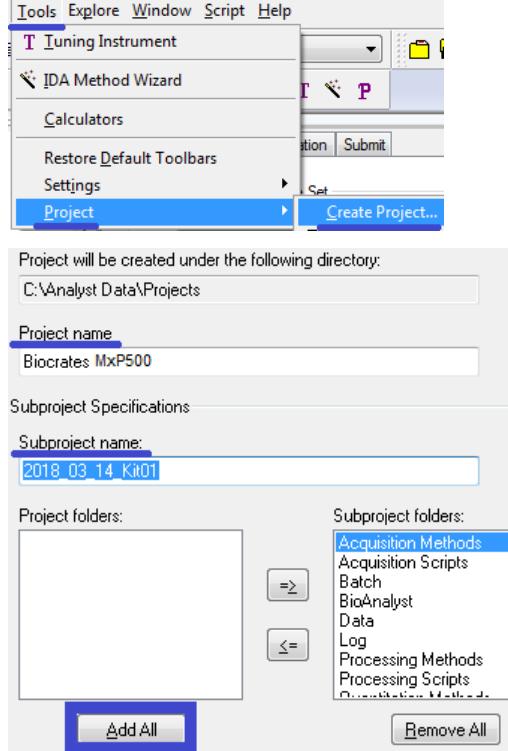
Column cleaning: It is recommended to clean the column after each plate for at least 30 min using the wash solvent (see section 5.4, page 34).

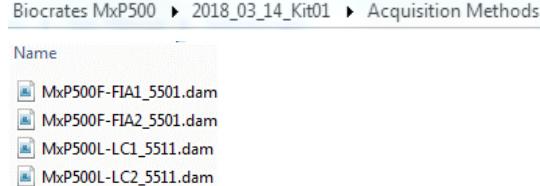
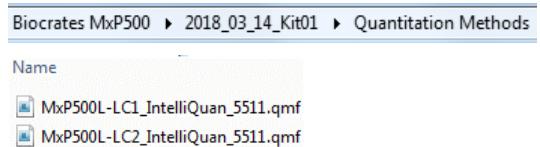
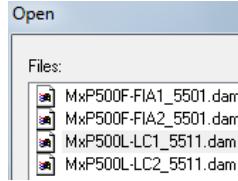
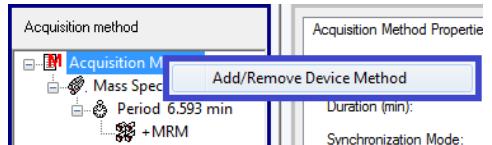
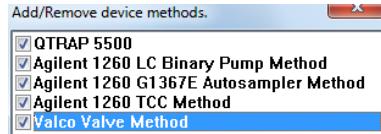
Column storage: 100% acetonitrile.

4.2 Acquisition Methods LC



Acquisition Methods are provided for each MS platform on the USB stick.
Update the *Acquisition Methods* for the used LC-MS combination.

Step	Instruction	Example
1	On the PC that operates the SCIEX instrument (hereinafter <i>MS-PC</i>) start Analyst®.	
2	<p>Create a new Analyst® project, subplot, and add all <i>Project folders</i>, e.g.</p> <ul style="list-style-type: none"> - project name: <i>Biocrates MxP500</i> - subplot: <i>2018_03_14_Kit01</i> <p><i>Information:</i> this project name will be used in the following instructions.</p>	

Step	Instruction	Example
3	<p>Use the <i>Windows-Explorer</i> to open the subproject folder “2018_03_14_Kit01”.</p> <p>Copy all required acquisition methods</p> <ul style="list-style-type: none"> - into the folder “Acquisition Methods” <p>Copy the quantitation method</p> <ul style="list-style-type: none"> - into the folder “Quantitation Methods” <p>from the USB stick according to the tables in section 4.</p>	 
4	Open the acquisition method <i>MxP500L-LC1_5511.dam</i> .	
5	To add autosampler, pump, and column oven, right-click on “Acquisition Method” and select “Add/Remove Device Method”.	
6	<p>Add all available devices.</p> <p><u>Note:</u> To the right an example is shown.</p> <p>The specific equipment listed will vary.</p>	
7	Check the “Synchronization Mode”: “LC Sync” is required.	<p>Synchronization Mode: <input type="button" value="LC Sync"/></p>
8	For the <i>Acquisition Method</i> use the parameters listed in sections 11.1 and 11.2.	 Refer to sections 11.1 and 11.2
9	 Repeat steps 4 to 8 with the acquisition method <i>MxP500L-LC2_5511.dam</i>.	

4.3 Acquisition Methods LC SST



An *Acquisition Method* for the LC system suitability test (SST) is not provided on the USB stick.

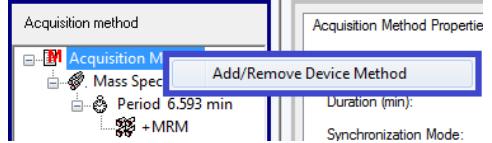
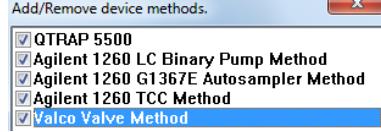
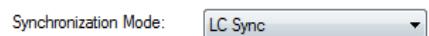
Step	Instruction	Example
1	Open the acquisition method <i>MxP500-LC1_5511.dam</i> .	
2	Activate the MS part of the method.	
3	Define a <i>MRM detection window</i> of 120 sec.	MRM detection window: <input type="text" value="120"/> (sec)
4	Define a <i>Target Scan Time</i> of 0.15 sec.	Target Scan Time: <input type="text" value="0.15"/> (sec)
5	Check the “Synchronization Mode”: “LC Sync” is required.	Synchronization Mode: <input type="button" value="LC Sync"/>
6	Save the Method as <i>MxP500-LC1_5511_adjustRT.dam</i> .	
	Ignore the Analyst® warning, that some MRM windows may exceed the method duration time.	
7	To create the SST acquisition method <i>MxP500-LC2_5511_adjustRT.dam</i> , repeat steps 1 to 6 with the acquisition method <i>MxP500-LC2_5511.dam</i> .	

4.4 Acquisition Methods FIA



Acquisition Methods are provided for each MS platform on the USB stick.

Update the *Acquisition Methods* for the used LC-MS combination.

Step	Instruction	Example
1	Open the FIA acquisition method <i>MxP500F-FIA1_5501.dam</i> .	
2	To add autosampler, pump, and column oven right-click on "Acquisition Method".	
3	Add all available devices. <u>Note:</u> to the right an example is shown.	
4	Check the "Synchronization Mode": "LC Sync" is required.	
5	For the <i>Acquisition Method</i> use the parameters shown in section 11.1 and 11.2.	 Refer to sections 11.1 and 11.2
6	 Repeat steps 1 to 5 with the acquisition method <i>MxP500F-FIA2_5501.dam</i>.	

5 Preparing Solvents



Follow lab safety procedures.

Use fume hood and gloves.

Dispose organic solvents properly.

Safety data sheets (SDS) are provided for kit components on the USB stick, located in the folder “MxP 500\Documents and Notes\SDS”. In addition, SDS are available on request. For contact details please refer to page 130.



Safety data sheets (SDS) are provided on the USB stick (folder “MxP 500\Documents and Notes\SDS”) and on request. For contact details please refer to page 130.



Test Samples are provided with each kit. Perform system suitability tests (SST) for the LC and FIA part with the Test Samples in order to check the instrument performance **before** starting with the kit preparation. See section 6 *System Suitability Test (SST)* for instructions.

If the SST fails, do not start with the kit preparation! Otherwise you will not be able to analyze your samples reliably and may lose sample information. Perform troubleshooting and contact Biocrates® customer support if necessary. For technical support by Biocrates®, the Test Sample data files are required.

5.1 Preparing Mobile Phases and Solvents



Wash all solvent bottles before use at least with water, isopropanol, methanol, and finally with the main component of the solvent.

E.g. solvent B: wash with water, isopropanol, methanol, and acetonitrile.

5.2 LC Part – Solvents A and B

<i>Mobile Phase</i>	<i>Description</i>
Solvent A (2 000 mL)	2 000 mL water + 4 mL formic acid
Solvent B (2 000 mL)	2 000 mL acetonitrile + 4 mL formic acid

5.3 FIA Part – FIA Solvent

Follow the instructions below to open the glass ampule (FIA Mobile Phase Additive) and to prepare the FIA solvent. Use freshly prepared solvents only. The second ampule (spare ampule) is provided in case of a mistake being made or a rerun of the kit plate being required.

Step	Instruction	Example
1	Have the white dot above the neck of the ampule facing you.	
2	Use glass-handling safety gloves when breaking the ampule open. Hold the ampule upright in one hand, then grip the top of the ampule firmly between the thumb and forefinger, placing your thumb on the white dot. Snap off the top of the ampule by bending it sharply backwards.	
3	Mix the content of one ampule with 290 mL of methanol to make the FIA solvent. FIA Solvent (300 mL) = 290 mL methanol + 1 ampule FIA Mobile Phase Additive	
	Empty ampules can be handled as common glass waste. Do not recycle empty ampules.	

5.4 Autosampler Wash Solvents

Prepare at least 1 000 mL of each solvent for one Kit.

Solvent	Description
Wash Solvent 1	25% water, 25% methanol, 25% acetonitrile, 25% isopropanol, <i>500 mL water, 500 mL methanol, 500 mL acetonitrile, 500 mL isopropanol</i>
Wash Solvent 2 — if possible —	LC part: 100% water <i>1 000 mL water</i> FIA part: 33% methanol, 33% isopropanol, 33% water <i>333 mL methanol, 333 isopropanol, 333 mL water</i>
Seal Wash	As recommended by the manufacturer,e.g. 10% <i>methanol</i> , 90 % <i>water</i>

6 System Suitability Test (SST)

The System Suitability Test (SST) is used to check the UHPLC-MS system performance **before** starting with the preparation of the MxP® Quant 500 Kit. Use the system settings described in this section. All required instrument method parameters are shown in section 4. Follow the instructions for your LC-MS instrumental setup.

LC-MS instrument method parameters:



- 4.1 Autosampler, Column Oven, and Pump Settings
- 4.2 Acquisition Methods LC
- 4.3 Acquisition Methods LC SST
- 4.4 Acquisition Methods FIA



If the SST fails, do not start with the kit preparation. Perform troubleshooting or contact Biocrates® Customer Support. The system may not be sensitive enough to detect all metabolites, especially in the concentration range of lower calibration standard levels.



The mass spectrometer must meet all manufacturer specifications and must be recently **tuned and calibrated for positive and negative ion mode**.

The autosampler must be suitable for 1 mL 96 deep well plates.

6.1 Cleaning of LC-MS System and Installation of Solvents



Clean the entire LC-MS system before using the MxP® Quant 500.

Step	Instruction
1	Thoroughly clean the ion source, e.g. <i>curtain plate, ESI electrode</i> .
2	<p>Install all wash solvents and prime.</p> <p>Wash solvent 1: 25% acetonitrile, 25% methanol, 25% isopropanol, 25% water</p> <p>Wash solvent 2: LC part: 100% water. – if possible – FIA part: 33% methanol, 33% isopropanol, 33% water</p> <p> If it is not possible to install two wash solvents, use solvent 1.</p>
3	Install all solvents (solvent A, solvent B, FIA solvent) and purge the lines.
4	Flush all LC capillaries using all three solvents.

6.2 Preparation of Blank, Test Samples, and Water Addition



Two Test Sample vials are provided with each kit, one for the LC part and one for the FIA part. The Test Sample vials are ready-to-use human plasma-based samples containing internal standards. The Test Sample LC contains all metabolites that are measured in the LC part. The Test Sample FIA contains typical metabolites of the FIA part. The SST must be passed successfully before using the kit.

<i>Item</i>	<i>Preparation</i>
Blank LC	Add 1000 µL of 50% methanol in water to an empty vial.
Test Sample LC	<ol style="list-style-type: none"> 1. Add 500 µL of <u>methanol</u> to the vial <i>Test Sample LC</i>. 2. Vortex for 10 sec. 3. Shake for 15 min at 600 rpm. 4. Add 500 µL of <u>water</u>. 5. Vortex for 10 sec.
Blank FIA	Add 1000 µL of FIA solvent to an empty vial.
Test Sample FIA	<ol style="list-style-type: none"> 1. Add 1000 µL of FIA solvent to the vial <i>Test Sample FIA</i>. 2. Vortex for 10 sec. 3. Shake for 15 min at 600 rpm. 4. Vortex for 10 sec.
Water addition vials	<ol style="list-style-type: none"> 1. Add 1.5 mL water to two empty vials. 2. Place the vials in autosampler according to section 11.1.4. <p style="text-align: center;"> Refer to section 11.1.4</p>



Dissolve Test Samples shortly before use. At 4 °C the stability of dissolved Test Samples is at least 1 week. If stored for longer periods, at 4 °C, metabolites may degrade.

Note: after two days Sarcosine is degraded, and intensities of other analytes are reduced.

6.3 Conditioning the UHPLC Column

Step	Instruction
1	Install the analytical column and flush the column for 20 min with 95% solvent B at a flow rate of 0.5 mL/min.
2	Before the first injection, equilibrate the system at starting conditions: 100% solvent A, flow rate 0.8 mL/min, column oven temperature 50 °C
3	Perform the SST (see next section).
i	If the SST fails due to insufficient cleaning, indicated by e.g. contaminations in blank chromatograms, perform the following steps.
4	Calibrate the instrument.
5	Change all tubings, if possible.
6	Install a new ESI electrode, if required.

6.4 Performing the System Suitability Test (SST)

The System Suitability Test (SST) is used to check the UHPLC-MS system performance before the MxP® Quant 500 is prepared. Use each method with the parameters as described in sections 11.1 and 11.2.



ESI probe position:

		5500 series
LC part	x-axis	5
	y-axis	5
FIA part	x-axis	5
	y-axis	5



Perform the SST before starting the kit preparation. If the SST fails, do not start with the Kit preparation and perform troubleshooting! If required, contact SCIEX or Biocrates® customer support.

Use the appropriate acquisition methods for your SCIEX instrument.

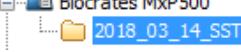
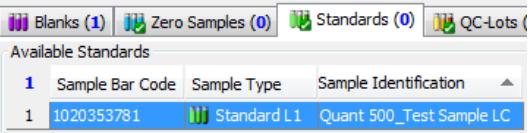
<i>MS Instrument</i>	5500 series
<i>LC variant</i>	UHPLC
<i>LC methods</i>	MxP500L-LC1_5511_adjustRT.dam MxP500L-LC2_5511_adjustRT.dam
<i>FIA methods</i>	MxP500F-FIA1_5501.dam MxP500F-FIA2_5501.dam

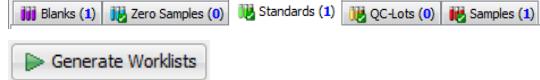
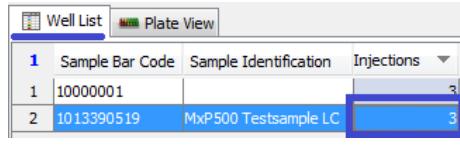
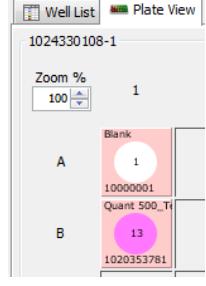
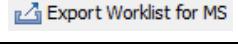
6.4.1 Adjust Retention Times (RTs)

Use the blank **LC** and Test Sample **LC** prepared in section 6.2 to adjust the RTs.

For this procedure a MetIDQ™ worklist is used.

Create MetIDQ™ worklist

Step	Instruction	Example
i	For more details who to create a plate and worklist, refer to the MetIDQ™ Nitrogen user manual section 4.1.4. Generate Plate Layout and Worklist for MS run.	
1	Go to MetLIMS > Projects .	
2	Create or use a MetIDQ™ project, e.g. - project name: <i>Biocrates MxP500</i> Create a MetIDQ™ submission, e.g. - subproject: <i>2018_03_14_SST</i>	
3	Link any sample, available in the database, in this example “Sample 2017-01”.	
4	Generate a new Worklist. Use OP <i>MXP500L-0-5511</i> .	
5	In the tab Zero Samples , cancel the selection of zero samples.	
6	From the tab Standards , link the standard “Quant 500_Test Sample LC”.	

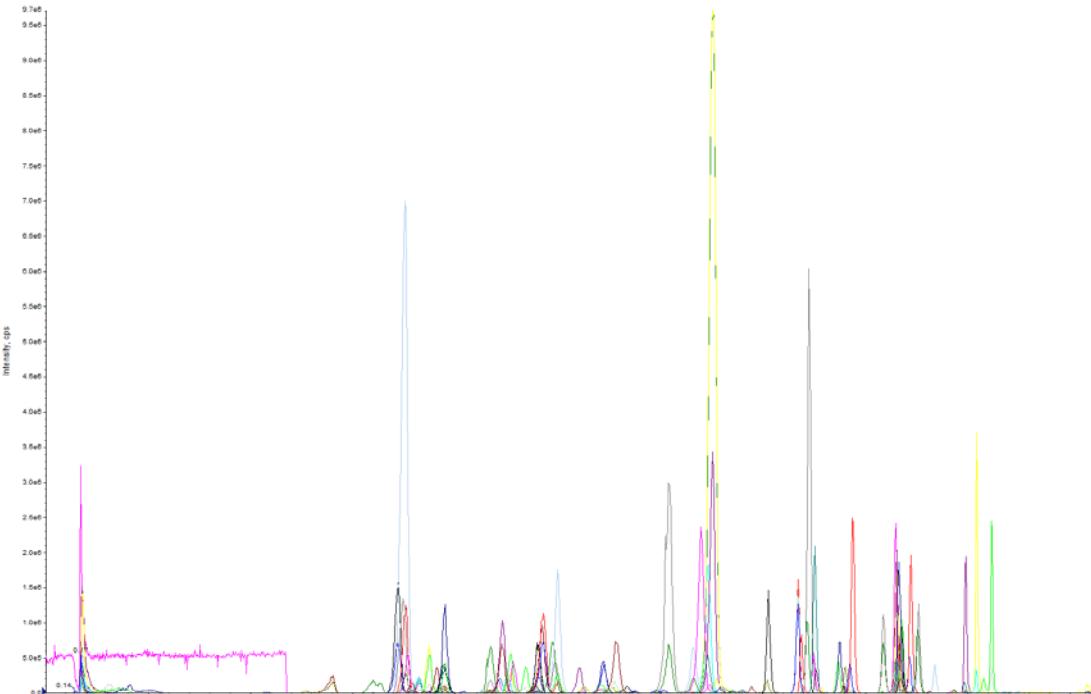
Step	Instruction	Example
7	Do not link additional samples. Click Generate Worklist .	
8	From the tab Plate View , delete the linked sample, in this example "Sample 2017-01".	
9	In the "Well List", define "Injections = 3" for blank and Test Sample LC.	
10	Now a plate is registered consisting of a blank and a Test Sample LC.	
11	Create a Worklist for MS.	

Run blank LC and Test Sample LC

Step	Instructions
12	Place the blank LC and Test Sample LC vials in the cooled autosampler tray. blank LC → position 1 Test Sample LC → position 2
13	Start Analyst® and build a new acquisition batch: File > New > Acquisition Batch.

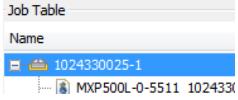
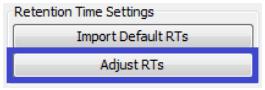
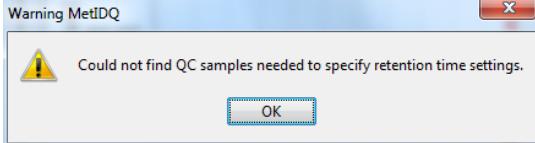


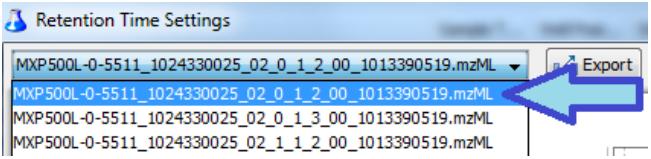
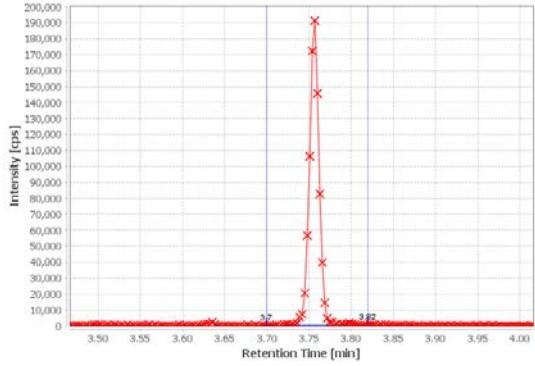
Step	Instructions
14	<p>Create a new acquisition batch: Import the .csv file from step 8 (created by MetIDQ™) into Analyst®. Follow the steps below:</p> <ol style="list-style-type: none"> 1. Right click on the New Batch window. 2. Select Import From > File. 3. Change the file type to .csv. 4. Select the MetIDQ™ worklist export (.csv file) and click Open.  <p>File type: Microsoft Text Driver (*.txt; *.csv)</p>
	<p>For injections from a Test Sample vial, change the “Plate Code” to “54VialPlate” for <u>both</u> sets.</p> 
15	<p>Check that the acquisition methods <i>MxP500L-LC1_5511_adjustRT.dam</i> and <i>MxP500L-LC2_5511_adjustRT.dam</i> are selected.</p> 
	<p>To identify the RT of each metabolite, acquisition methods with the name extension “_adjustRT” have 2 min RT windows.</p>
	<p>Two “Sets” are used to perform injections with the acquisition methods LC1 and LC2. Check the “Plate Code” for <u>both</u> sets.</p> 
16	<p>Define the correct <i>Rack Code</i>, <i>Rack Position</i>, <i>Plate Code</i>, <i>Plate Position</i>, and <i>Vial Position</i>.</p>
	<p>Do not rename or alter the samples, data files, or acquisition methods. Otherwise the data files will not be compatible with MetIDQ™.</p>
17	<p>Submit the sequence and equilibrate the LC-MS system.</p> 
	<p>Before starting an injection, wait until all LC-MS parameters are stable. The blank sample will be injected three times to condition the system. Double-check that two vials for “water addition” are in the autosampler, see Table 1.</p>
18	<p>Run the batch.</p> 

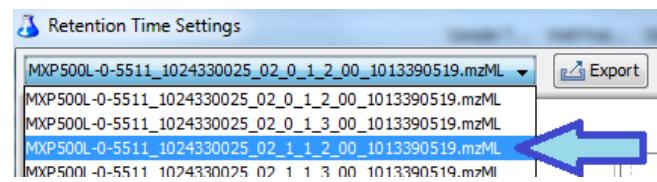
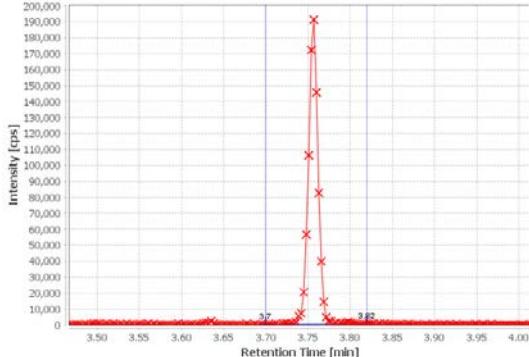
Step	Instructions
1	<p>Figure 1 shows an example chromatogram of a Test Sample LC injection.</p> <p>MS instrument: SCIEX QTRAP® 5500</p> <p>Pump system: Agilent® 1290</p> <p>Acquisition method: MxP500L-LC1_5511_adjustRT.dam</p>  <p><i>Figure 1: Example LC Test Sample chromatogram measured with SCIEX 5500 + Agilent® 1290.</i></p>

Identify RTs

Peak integration and quantitation is performed by Met/DQ™. Test Sample LC injections are used to adjust the RTs.

Step	Instruction	Example
i	For additional information, refer to the Met/DQ™ Nitrogen user manual section 5.1 <i>LC data – quantitation by Met/DQ™</i> .	
19	Go to MetCONC > Import Raw Files .	
20	Select the “plate” used for the SST. <u>Note:</u> blank and Test Sample LC may be injected from vials.	
21	Click Adjust RTs .	
22	Ignore this warning. Click OK .	

Step	Instruction	Example
23	<p><u>RT adjustment positive mode:</u></p> <p>Select a representative Test Sample LC chromatogram, positive mode.</p> <p>To identify the Test Sample use the acquisition method number and barcode included in each file name,</p> <p>e.g. MxP500L-0-5511_102433025_02_0_1_2_00_1013390519.mzML</p> <p>0 = positive mode 1 = negative mode</p> 	
	  For peak identification, refer to section 9.2 <i>Peak Identification</i> .	
24	Adjust the integration ranges for all metabolites acquired in <u>positive</u> mode according to the MetIDQ™ user manual, section 5.1 <i>LC data – quantitation by MetIDQ™</i> .	
25	Export the adjusted RTs, e.g. <i>RT_export_pos_MxP500.xlsx</i>	

Step	Instruction	Example
26	<p><u>RT adjustment negative mode:</u></p> <p>Select a representative Test Sample LC chromatogram, negative mode. E.g.,</p> <p>e.g. MxP500L-0-5511_102433025_02_1_1_2_00_1013390519.mzML</p> 	
27	<p>Adjust the integration ranges for all metabolites acquired in <u>negative</u> mode according to the MetIDQ™ user manual, section 5.1 <i>LC data – quantitation by MetIDQ™</i>.</p>	
	 For peak identification, refer to section 9.2 <i>Peak Identification</i> .	
28	Export the adjusted RTs, e.g. <i>RT_export_neg_MxP500.xlsx</i>	
29	Close the “Retention Time Settings” window.	

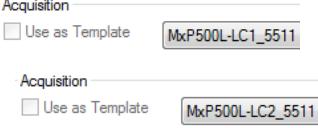
Adjust RTs in acquisition methods

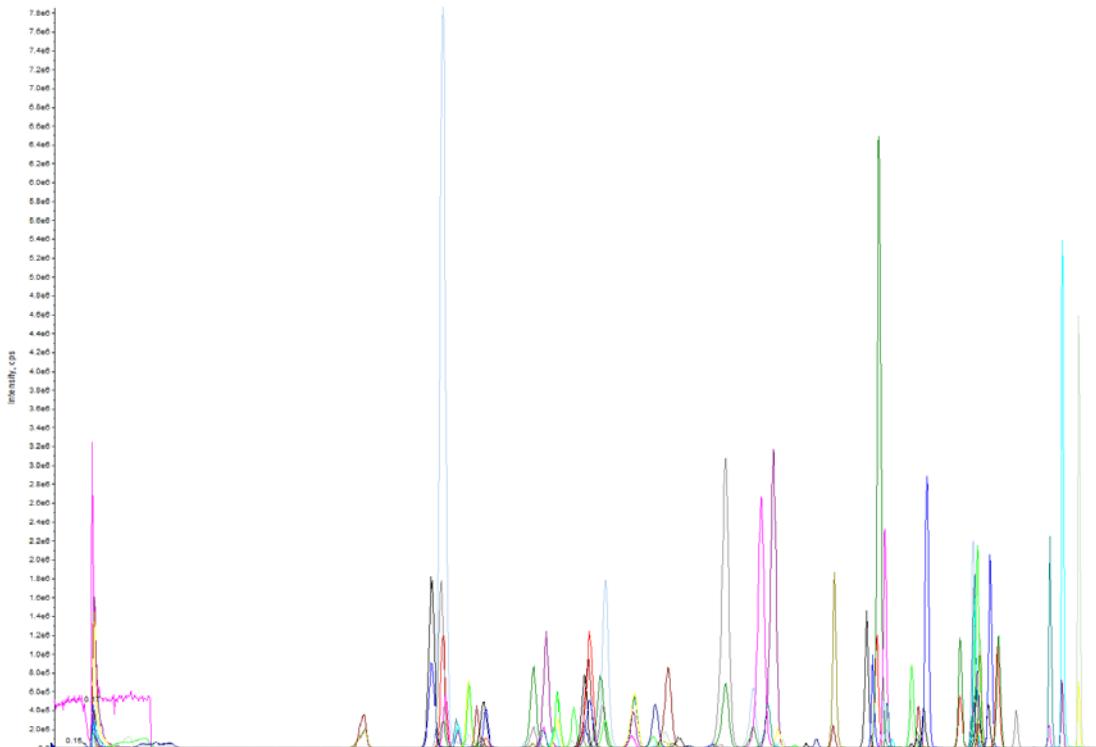
For the SST and kit runs, adjust the RTs in the acquisition methods.

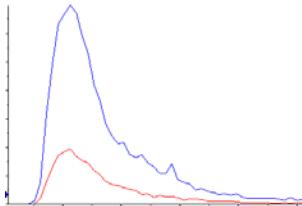
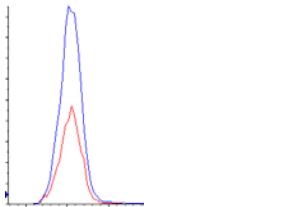
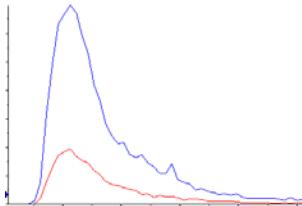
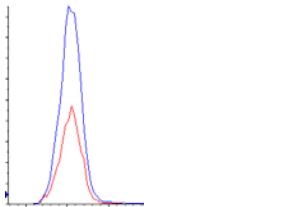
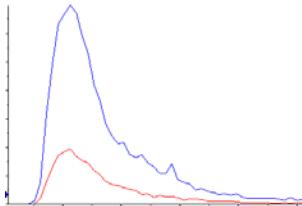
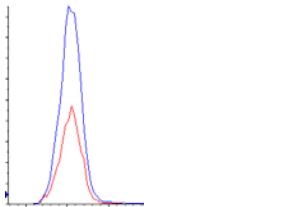
Step	Instruction	Example															
30	Open the acquisition method <i>MxP500L-LC1_5511.dam</i> with Analyst®.																
31	Adjust <u>all</u> RTs according to the Test Sample LC chromatograms, refer to the recently saved file <i>RT_export_pos_MxP500.xlsx</i> .	<table border="1"> <thead> <tr> <th>Q3 Mass (Da)</th> <th>Time (min)</th> <th>ID</th> </tr> </thead> <tbody> <tr> <td>6.0</td> <td>2.17</td> <td>ADMA</td> </tr> <tr> <td>0.200</td> <td>2.23</td> <td>D6-SDMA-PTC</td> </tr> <tr> <td>0.100</td> <td>2.24</td> <td>SDMA</td> </tr> <tr> <td></td> <td></td> <td>71</td> </tr> </tbody> </table>	Q3 Mass (Da)	Time (min)	ID	6.0	2.17	ADMA	0.200	2.23	D6-SDMA-PTC	0.100	2.24	SDMA			71
Q3 Mass (Da)	Time (min)	ID															
6.0	2.17	ADMA															
0.200	2.23	D6-SDMA-PTC															
0.100	2.24	SDMA															
		71															
!	Metabolites and internal standards have the same RT.																
32	Save the changes.																
33	Open the acquisition method <i>MxP500L-LC2_5511.dam</i> with Analyst®.																
34	Adjust <u>all</u> RTs according to the Test Sample LC chromatograms, refer to the recently saved file <i>RT_export_neg_MxP500.xlsx</i> .	<table border="1"> <thead> <tr> <th>Q3 Mass (Da)</th> <th>Time (min)</th> <th>ID</th> </tr> </thead> <tbody> <tr> <td>43.3</td> <td>2.19</td> <td>CA</td> </tr> <tr> <td>4.000</td> <td>2.25</td> <td>GCDCA</td> </tr> <tr> <td>4.000</td> <td>2.33</td> <td>GnGCA</td> </tr> <tr> <td></td> <td></td> <td>-1</td> </tr> </tbody> </table>	Q3 Mass (Da)	Time (min)	ID	43.3	2.19	CA	4.000	2.25	GCDCA	4.000	2.33	GnGCA			-1
Q3 Mass (Da)	Time (min)	ID															
43.3	2.19	CA															
4.000	2.25	GCDCA															
4.000	2.33	GnGCA															
		-1															
!	Metabolites and internal standards have the same RT.																
35	Save the changes.																

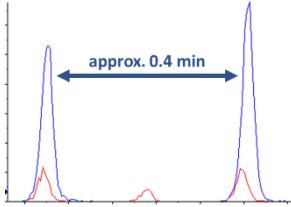
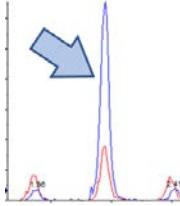
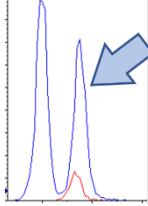
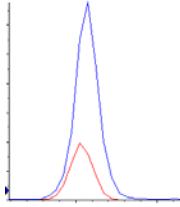
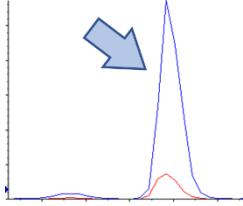
6.4.2 SST – LC Part

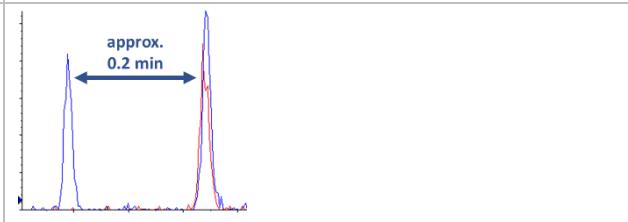
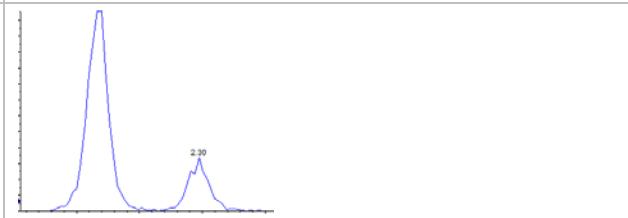
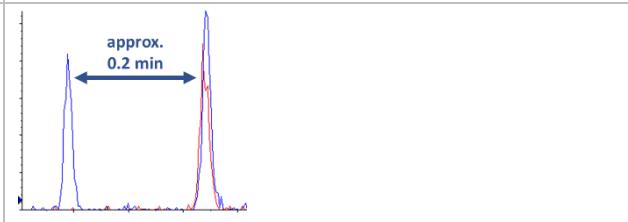
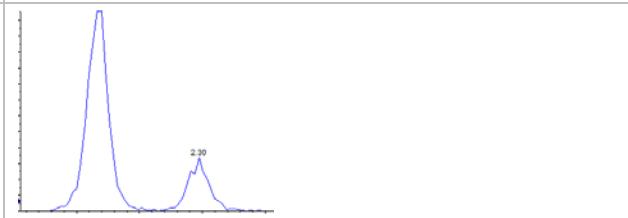
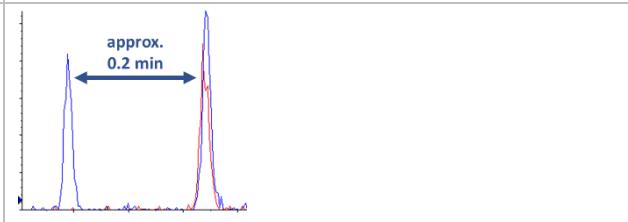
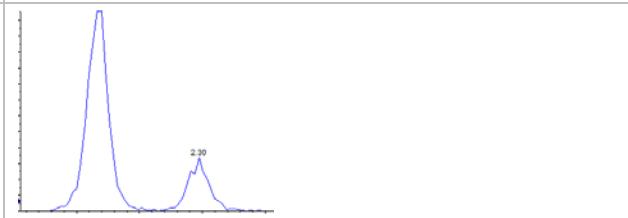
Use the blank **LC** and Test Sample **LC** vials prepared in section 6.2 for the SST.

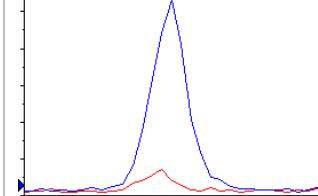
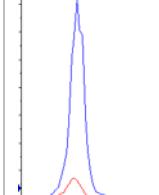
Step	Instruction
1	Use the acquisition methods <i>MxP500L-LC1_5511.dam</i> and <i>MxP500L-LC2_5511.dam</i> .
	Before starting an injection wait until all LC-MS parameters are stable.
2	Place the blank LC and Test Sample LC vials into the cooled autosampler tray.
3	Create a sample sequence: - 3x blank LC using <i>MxP500L-LC1_5511.dam</i> - 3x Test Sample LC using <i>MxP500L-LC1_5511.dam</i> - 3x Test Sample LC using <i>MxP500L-LC2_5511.dam</i> - 2x blank LC using <i>MxP500L-LC1_5511.dam</i>
	Injection volume: 5 µL
4	In the <i>Batch Editor</i> activate “Use Multiple Methods”. <input checked="" type="checkbox"/> Use Multiple Methods
5	Use the appropriate methods, <i>MxP500L-LC1_5511.dam</i> and <i>MxP500L-LC2_5511.dam</i> . 
6	Define the correct <i>Rack Code</i> , <i>Rack Position</i> , <i>Plate Code</i> , <i>Plate Position</i> , and <i>Vial Position</i> .
7	Submit and start the sequence. <input type="button" value="Submit"/> >  > 
8	The retention times (RT) of each analyte must be stable over all three LC Test Sample injections. If not, perform troubleshooting according to Biocrates® online FAQ, section LC Troubleshooting .

Step	Instruction
9	<p>Figure 2 shows an example chromatogram of a LC Test Sample injection.</p> <p>MS instrument: SCIEX QTRAP® 5500</p> <p>Pump system: Agilent® 1290</p> <p>Acquisition method: MxP500L-LC1_5511.dam</p>  <p>Figure 2: Example Test Sample LC chromatogram measured with SCIEX 5500 + Agilent® 1290.</p>
FAQ	<p>Biocrates® FAQ: https://support.biocrates.com/tiki-index.php</p>

Step	Instruction						
	<p>To evaluate the LC performance, use these SST criteria.</p> <p>i For this procedure use Analyst®. Analyte (blue) and corresponding internal standard (red) is shown.</p> <p>For LC1 methods <i>MxP500L-LC1_5511.dam</i></p> <p>SST criteria – all metabolites:</p> <ul style="list-style-type: none">• Blank chromatograms: between 1.5 and 4.0 min background noise level below 2.5e4 cps.• Stable RTs over three LC Test Sample injections, maximum tolerance ± 0.03 min• Backpressure profile should not show any ripples <p>SST criteria – example metabolites:</p> <table border="1"><thead><tr><th>Metabolite</th><th>Example chromatogram</th></tr></thead><tbody><tr><td>Creatinine:</td><td></td></tr><tr><td>Taurine:</td><td></td></tr></tbody></table>	Metabolite	Example chromatogram	Creatinine:		Taurine:	
Metabolite	Example chromatogram						
Creatinine:							
Taurine:							
10							

Step	Instruction
	<p><u>t4-OH-Pro / c4-OH-Pro:</u></p> <ul style="list-style-type: none"> • good peak separation 
	<p><u>Asn:</u></p> <ul style="list-style-type: none"> • good detectability 
	<p><u>SDMA:</u></p> <ul style="list-style-type: none"> • good peak separation 
	<p><u>Leu:</u></p> <ul style="list-style-type: none"> • no fronting, no tailing 
	<p><u>Spermine:</u></p> <ul style="list-style-type: none"> • good detectability 

Step	Instruction								
	<p>For LC2 methods <i>MxP500L-LC2_5511.dam</i></p> <p>SST criteria – all metabolites:</p> <ul style="list-style-type: none"> • Blank chromatograms: between 0.0 and 3.0 min background noise level below 2.5e4 cps. • Stable RTs over three LC Test Sample injections, maximum tolerance ± 0.03 min • Backpressure profile should not show any ripples <p>SST criteria – example metabolite:</p> <table border="1" data-bbox="287 584 1402 1294"> <thead> <tr> <th data-bbox="287 584 774 647">Metabolite</th><th data-bbox="774 584 1402 647">Example chromatogram</th></tr> </thead> <tbody> <tr> <td data-bbox="287 647 774 854"><u>p-Cresol-SO₄:</u></td><td data-bbox="774 647 1402 854">  </td></tr> <tr> <td data-bbox="287 854 774 1076"><u>TMCA / TCA:</u></td><td data-bbox="774 854 1402 1076">  </td></tr> <tr> <td data-bbox="287 1076 774 1294"><u>GCDCA / GDCA:</u></td><td data-bbox="774 1076 1402 1294">  </td></tr> </tbody> </table>	Metabolite	Example chromatogram	<u>p-Cresol-SO₄:</u>		<u>TMCA / TCA:</u>		<u>GCDCA / GDCA:</u>	
Metabolite	Example chromatogram								
<u>p-Cresol-SO₄:</u>									
<u>TMCA / TCA:</u>									
<u>GCDCA / GDCA:</u>									

Step	Instruction
	<p><u>FA(12:0):</u></p> <ul style="list-style-type: none"> • no fronting, no tailing 
	<p><u>FA(18:0):</u></p> <ul style="list-style-type: none"> • no fronting, no tailing 
11	<p><u>Check autosampler settings for injections from 96 deep well plates (first Kit use only):</u></p> <ul style="list-style-type: none"> • Transfer 100 µL from the Test Sample LC vial into well positions A1 and H12 of an empty 1 mL deep well plate, provided with the first kit order. • Make one injection from both well positions A1 and H12 using the acquisition method “MxP500L-LC1_5511.dam”. • Compare the obtained chromatograms with the ones acquired during the LC1 SST. • All peak intensities should be comparable to the ones from the LC1 SST. <ul style="list-style-type: none"> ➤ If the peak intensities are reduced, it is possible that less than the specified sample volume was injected. Check the autosampler settings described in section 4.1.1.

Step	Instruction
✓	If the SST meets the required criteria <u>continue</u> with the SST for the FIA part.
✗	If the SST fails, the system may not be sensitive enough to detect all metabolites. Double-check the LC-MS configuration, instrument method parameters, and clean the entire LC-MS system according to the chapters 4 and 5. Perform the SST again. Please feel free to contact the Customer Support whenever you have questions.
!	If the SST fails, do not start with the kit preparation! Otherwise you may not be able to analyze your samples reliably and may lose sample information. Perform troubleshooting and contact Biocrates® customer support.

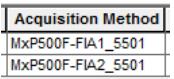
6.4.3 SST – FIA Part

Use the appropriate acquisition methods for your SCIEX instrument.

MS Instrument	5500 series
FIA methods	MxP500F-FIA1_5501.dam MxP500F-FIA2_5501.dam

Use the blank **FIA** and Test Sample **FIA** vials prepared in section 6.2 for the SST.

Step	Instruction
1	Use the acquisition methods <i>MxP500F-FIA1_5501.dam</i> and <i>MxP500F-FIA2_5501.dam</i> .
	Do not rename any method files!
2	Double-check all parameters in the acquisition method for all instrument parts (MS, pump, autosampler, column oven). If necessary, type in the correct parameters according to sections 11.1 and 11.2.
3	Prepare the autosampler for FIA by one of the following options: - Remove the column and, if possible, connect the injector directly with the ion source. <u>or</u> - Use a bypass or another column line (without <u>any</u> column installed).
	For good FIA peaks, minimize dead volumes by using as few connecting parts as possible.
4	Flush the system with the FIA solvent at a flow rate of 0.2 mL/min for 10 min.
5	Place the FIA blank vial and FIA Test Sample vial in the cooled autosampler tray.

Step	Instruction
6	Create a sample sequence: - 3x blank FIA - 3x Test Sample FIA using <i>MxP500F-FIA1_5501.dam</i> - 3x Test Sample FIA using <i>MxP500F-FIA2_5501.dam</i> - 2x blank FIA <u>Injection volume:</u> 20 µL
7	In the <i>Batch Editor</i> activate “Use Multiple Methods”. <input checked="" type="checkbox"/> Use Multiple Methods
8	Use the appropriate methods, <i>MxP500F-FIA1_5501.dam</i> and <i>MxP500F-FIA2_5501.dam</i> . 
9	Define the correct <i>Rack Code</i> , <i>Rack Position</i> , <i>Plate Code</i> , <i>Plate Position</i> , and <i>Vial Position</i> .
10	Submit and start the sequence.  >  > 

To evaluate the FIA Test Sample performance, Test Sample FIA SST chromatograms, show the TIC, and use the SST criteria of step 12.

Figure 3 and Figure 4 show example chromatograms of Testsample FIA injections using the following setup:

MS instrument: SCIEX QTRAP 5500

Pump system: Agilent® 1290

Acquisition method: Figure 3: MxP500F-FIA1_5501.dam

Figure 4: MxP500F-FIA2_5501.dam

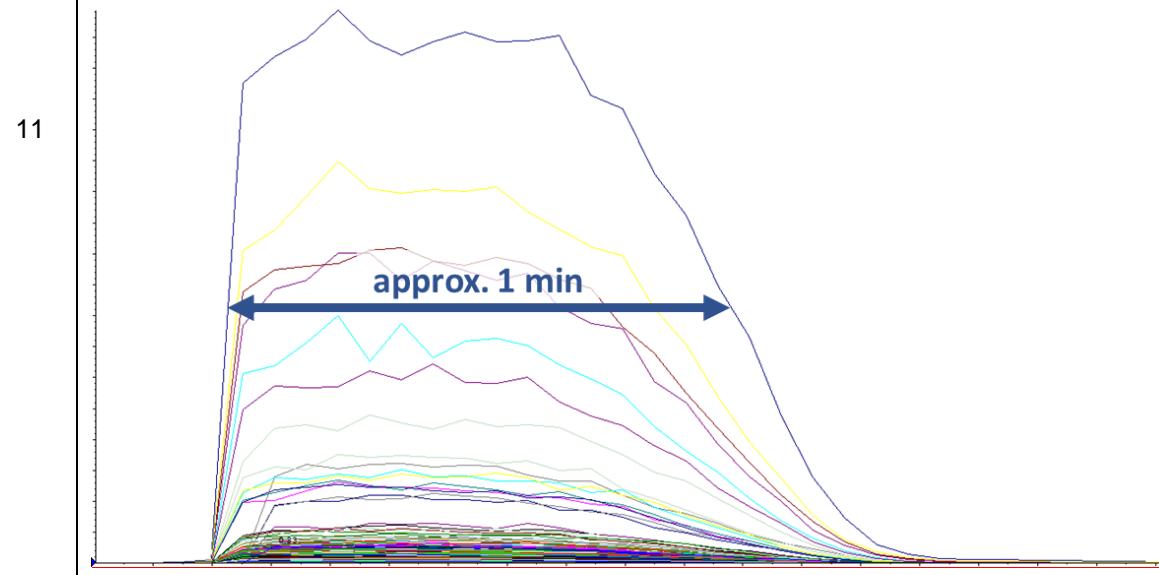
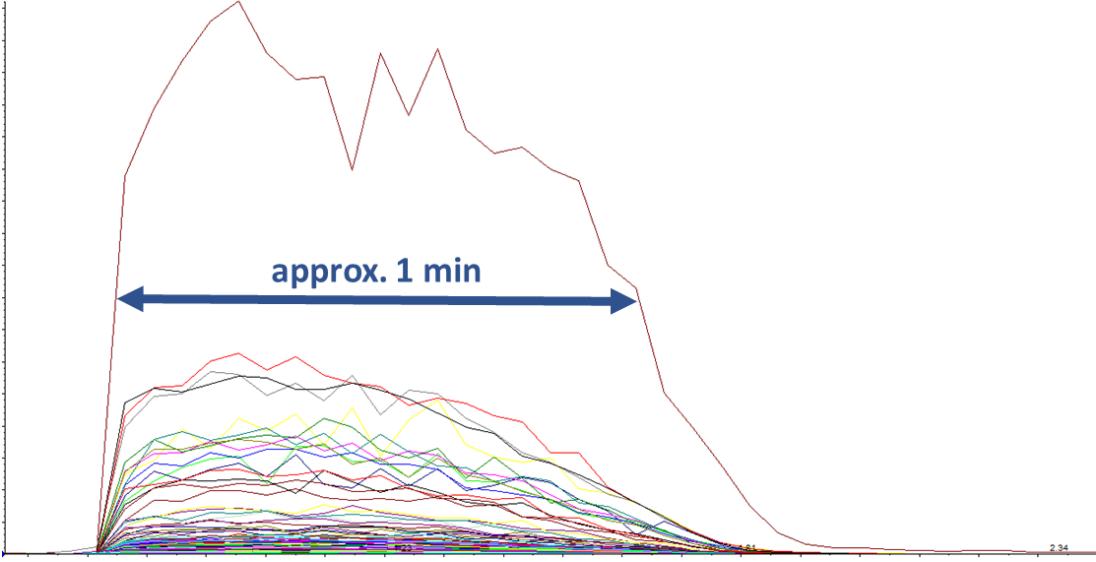
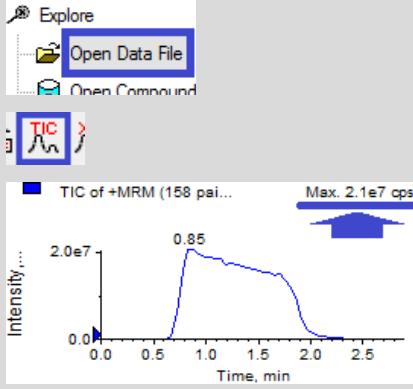


Figure 3: Example Test Sample FIA chromatogram measured with SCIEX 5500 + Agilent® 1290.

Step	Instruction
	
	<p><u>Open .wiff files and show TIC in Analyst®:</u></p> <ul style="list-style-type: none">- Start Analyst® and open a .wiff file.- Show the TIC.- The maximum intensity is shown in the upper right corner of the TIC screen. 

Step	Instruction				
12	<p>SST criteria – Test Sample:</p> <ul style="list-style-type: none">• Open a representative Test Sample FIA chromatogram and show the <i>T/C</i>.• The min. <i>T/C</i> intensity should be <table border="1" data-bbox="339 377 949 473"><tr><td data-bbox="339 377 632 417">FIA1</td><td data-bbox="632 377 949 417">FIA2</td></tr><tr><td data-bbox="339 417 632 473">5e7 cps</td><td data-bbox="632 417 949 473">2e7 cps</td></tr></table> <ul style="list-style-type: none">• Representative chromatograms are shown above, see Figure 3 and Figure 4. The FIA peak shape may depend on the used LC-MS combination. <p>FIA peak criteria:</p> <ul style="list-style-type: none">• Located approximately in the middle of the data acquisition window.• Defined beginning and ending, without huge valleys in between.• No “satellite“or huge shoulder peaks.• Stable ESI spray: no signal dips.	FIA1	FIA2	5e7 cps	2e7 cps
FIA1	FIA2				
5e7 cps	2e7 cps				

Step	Instruction
	<p>Troubleshooting:</p> <ul style="list-style-type: none"> • <u>Increased general background noise:</u> Clean LC-MS system, especially the tubings, valves, connecting parts, grounding <i>T-part</i>, the ESI electrode, and the cone. Make new FIA solvent using methanol from another batch or vendor. • <u>Peak in FIA blank:</u> Inject blank from a new blank vial. Clean the injection system, especially the needle and the needle seal. • <u>“Satellite“ peaks or huge shoulder peaks:</u> Retighten all connections. Avoid unnecessary valves in the flow path to the MS. • <u>Unstable ESI spray / signal dips:</u> Clean or replace the ESI electrode. Clean the grounding <i>T-part</i>. Replace the needle seal. Service syringe or injection valve, as it might be drawing air. • <u>Low sensitivity:</u> Clean and calibrate the MS instrument.
	<p>If the SSTs of LC <u>and</u> FIA parts meet the required criteria you may start with the kit preparation.</p>
	<p>If either the SST of the LC or FIA part fails, the system may not be sensitive enough to detect all metabolites.</p> <p>Double-check the LC-MS configuration, instrument method parameters, and clean the entire LC-MS system according to section 4. Perform the SST again.</p> <p>Please feel free to contact Biocrates® customer support whenever you have questions.</p>
	<p>If the SST fails, do not start with the Kit preparation! Otherwise, you may not be able to analyze your samples reliably and may lose sample information. Perform troubleshooting and contact Biocrates® customer support.</p>

7 Kit Preparation



The Met/DQ™ software is an integral part of the MxP® Quant 500. Please read the Met/DQ™ manual carefully (*UM-MetDQ-Nitrogen-#.pdf*) and install Oracle® and Met/DQ™ before starting with the kit preparation.

! Deactivate the Met/DQ™ option “Support Quantitation in Analyst” from the settings.
Note: if activated, worklist exports (.csv) are not compatible with Analyst®.

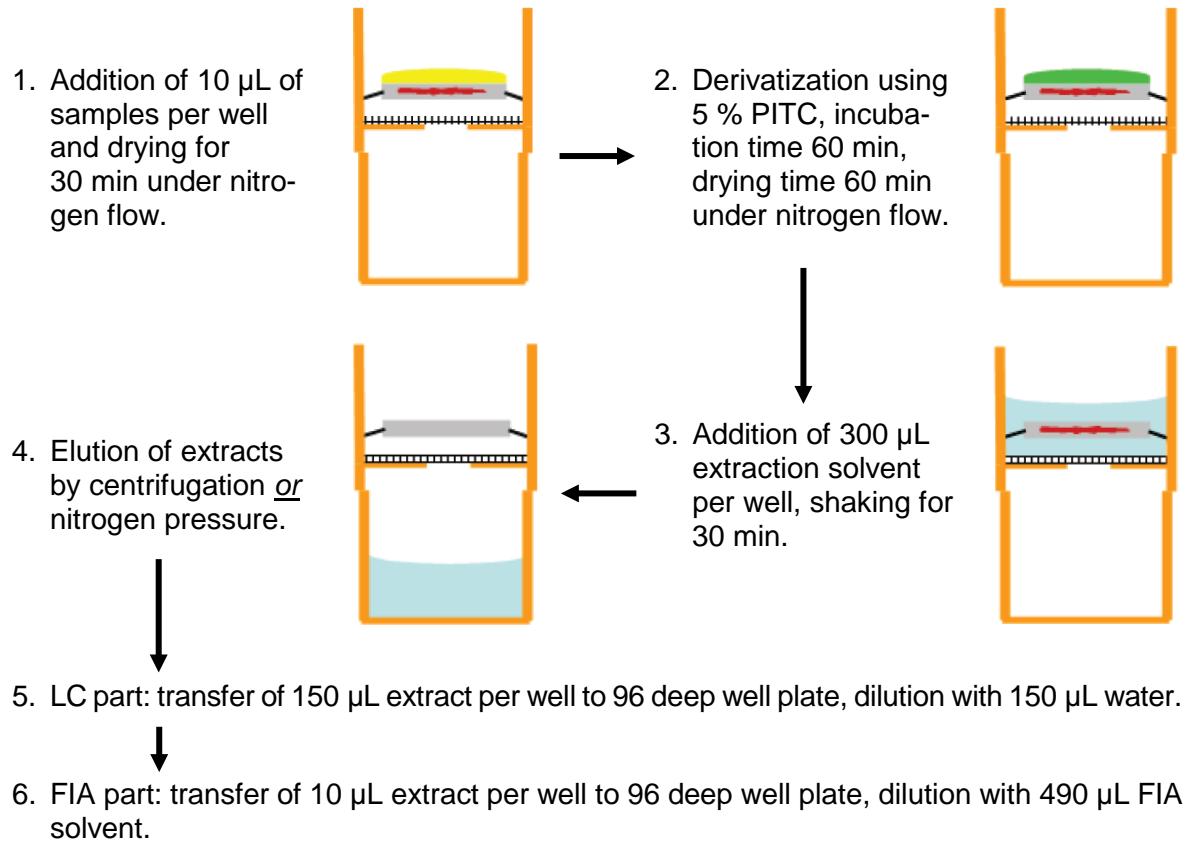
A screenshot of the Met/DQ software's settings window. On the left, there is a blue vertical bar with a white exclamation mark icon. To the right, there is a navigation path consisting of a flask icon followed by two arrows, then a gear icon followed by another arrow. The main window shows a toolbar with icons for General, MetLIMS, MetCONC, and MetVA. Below the toolbar, there are three tabs: General Settings (selected), SCIEX CSV Filename, and SCIEX WIFF File Path Settings. Under General Settings, there is a section for appending file names with an example: KIT3-0-5504_1013885985_38_0_1_1_03_1020158015_p180_QC1.wiff. At the bottom of this section is a checkbox labeled "Support Quantitation in Analyst:" which is checked. A small "SAM" label is visible next to the checkbox.

7.1 Overview Kit Workflow

The kit workflow is described in detail in the following sections. For proper kit performance, do not combine the kit with components from other manufacturers.



7.2 Overview Lab Workflow



7.3 Preparing Kit Components and Samples



Dissolve content of appropriate vials shortly before preparing the kit.

7.3.1 Phosphate Buffered Saline (PBS)

PBS and plasma have a similar salt composition, ion suppression, and background noise. Therefore, PBS is used as zero sample. PBS can also be used as zero sample when other matrices than plasma are analyzed. The limit of detection (LOD) for each metabolite is calculated by Met/DQ™. The LOD is defined as three times the background within zero samples.



While PBS is recommended for blood-based samples (e.g. plasma), for other matrices zero samples similar to the respective matrix can be used. In the case of tissue extracts, for example, the extraction solvent can serve as the zero sample.

Zero Sample	Instruction
PBS Solution	Prepare according to manufacturer's information.

7.3.2 Calibration Standards (Cal1 – Cal7)

Do not dissolve until use. **Centrifuge the vials before opening at 10 000 x g for 2 min.** The seven standards contain the amino acids and biogenic amines and are used to generate the calibration curves for the LC-MS part.

Standard	Instruction
Calibration standards	<ol style="list-style-type: none">1. Add 100 µL of water to each of the 7 calibration standards.2. Shake for 15 min at 1200 rpm and vortex several times.3. Gently tap the tubes on the table or use a centrifuge to make sure that the solution is at the bottom of the tube.

7.3.3 Quality Control Samples (QC1 – QC3)

Do not dissolve until use. **Centrifuge the vials before opening at 10 000 x g for 2 min.** The QC samples are human plasma spiked with several analytes in defined concentrations. QCs of three levels are provided.

Note: We recommend pipetting QC2 after every 20th samples

Sample	Instruction
QC Samples	<ol style="list-style-type: none">1. Add 100 µL of water to each QC vial.2. Vortex for 10 sec.3. Shake for 15 min at 1200 rpm.4. Vortex for 10 sec. <p> Do not centrifuge reconstituted QCs.</p>

7.3.4 Plasma Samples

Sample	Instruction
Plasma Samples	Vortex plasma samples after thawing.

7.4 Preparing Solvents and Reagents

7.4.1 Pre-Mix for Derivatization

Solution	Instruction
1900 µL ethanol 1900 µL water 1900 µL pyridine	<ol style="list-style-type: none">1. Pipette 1900 µL (or 2 x 950 µL) of each solvent into the empty plastic tube that you find in the kit box.2. Vortex for 10 sec.

7.4.2 Phenylisothiocyanate (PITC) Derivatization Solution



Prepare this solution directly before use!

Note: The stability of the solution is reduced after addition of PITC to the pre-mix.

Solution	Instruction
Derivatization Solution	<ol style="list-style-type: none">1. Remove the PITC from the freezer and allow it to equilibrate to room temperature.2. Add 300 µL of fresh PITC to the pre-mix.3. Vortex rigorously until the solution is clear.

7.4.3 Extraction Solvent

This solution is stable for approx. three months after preparation.

Solution	Instruction
5 mM ammonium acetate in <u>methanol</u>	Dissolve 19 mg ammonium acetate in 50 mL <u>methanol</u> .

7.4.4 Mobile Phases

For more details see sections

- 5.2 LC Part – Solvents A and B
- 5.3 FIA Part – FIA Solvent.



Wash all solvent bottles before use at least with water, isopropanol, methanol, and finally with the main component of the solvent.

E.g. solvent B: wash with water, isopropanol, methanol, and acetonitrile.

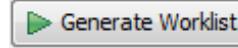
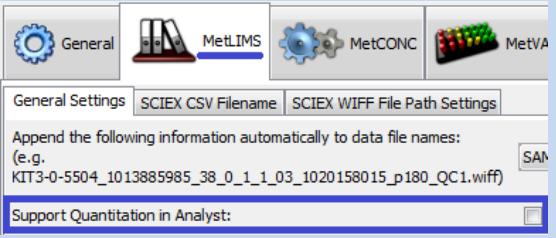
LC part:

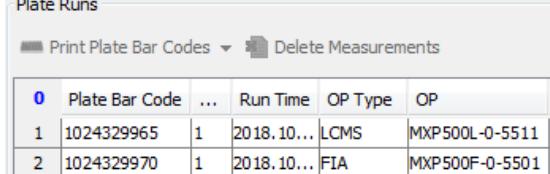
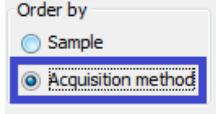
Mobile Phase	Description	
Solvent A (2000 mL)	2000 mL water	+ 4 mL formic acid
Solvent B (2000 mL)	2000 mL acetonitrile	+ 4 mL formic acid

FIA part:

Mobile Phase	Description
FIA Solvent (300 mL final vol.)	290 mL methanol + 1 ampule <i>FIA Mobile Phase Additive</i>

7.4.5 Registering the Kit Plate in Met/DQ™

Step	Instruction	Example												
1	To register the kit plate in Met/DQ™ follow the instructions of the Met/DQ™ Nitrogen user manual (<i>UM-MetIDQ-Nitrogen-#.pdf</i>) in section 4.1.4 <i>Generate Plate Layout and Worklist for MS run</i> .	 User Manual												
2	When generating the worklist in MetIDQ > MetLIMS > Projects , use the appropriate Met/DQ™ OPs shown in the tables in section 4.1.4 in the Met/DQ™ user manual.													
!	<p>Note: if activated, worklist exports (.csv) are not compatible with Analyst®.</p> <p>Deactivate the Met/DQ™ option “Support Quantitation in Analyst” from the settings.</p>													
i	<p>Two plate runs with different Met/DQ™ OPs are created in MetLIMS > Projects, the first for the LC and the second for the FIA part. An example is given for a SCIEX 5500 series instrument.</p> <p><u>Example for SCIEX 5500 series instrument – UHPLC:</u></p> <table border="1"> <thead> <tr> <th></th> <th>OP code</th> <th>Acquisition methods</th> <th>Quantitation methods</th> </tr> </thead> <tbody> <tr> <td>LC</td> <td>MXP500L-0-5511</td> <td>MxP500L-LC1_5511.dam MxP500L-LC2_5511.dam</td> <td>by Met/DQ™</td> </tr> <tr> <td>FIA</td> <td>MXP500F-0-5501</td> <td>MxP500F-FIA1_5501.dam MxP500F-FIA2_5501.dam</td> <td>by Met/DQ™</td> </tr> </tbody> </table>		OP code	Acquisition methods	Quantitation methods	LC	MXP500L-0-5511	MxP500L-LC1_5511.dam MxP500L-LC2_5511.dam	by Met/DQ™	FIA	MXP500F-0-5501	MxP500F-FIA1_5501.dam MxP500F-FIA2_5501.dam	by Met/DQ™	
	OP code	Acquisition methods	Quantitation methods											
LC	MXP500L-0-5511	MxP500L-LC1_5511.dam MxP500L-LC2_5511.dam	by Met/DQ™											
FIA	MXP500F-0-5501	MxP500F-FIA1_5501.dam MxP500F-FIA2_5501.dam	by Met/DQ™											

3	<p>1. Register one LC kit plate (<i>Plate Run</i>) using the OP “MXP500L-0-5511”.</p> <p>2. Once the LC plate layout is satisfactory, make one copy of this <i>Plate Run</i>.</p> <ul style="list-style-type: none"> - Right-click on the plate entry and select “Copy”. - Right-click again and “Paste derived plate” three times. <p>3. Change the OP of the newly created plate to the corresponding FIA OP, according to the table in section 4.1.4 in the Met/DQ™ user manual.</p>	 <p>Plate Runs</p> <p>Print Plate Bar Codes ▾ Delete Measurements</p> <table border="1"> <thead> <tr> <th>0</th> <th>Plate Bar Code</th> <th>...</th> <th>Run Time</th> <th>OP Type</th> <th>OP</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>1024329965</td> <td>1</td> <td>2018.10...</td> <td>LCMS</td> <td>MXP500L-0-5511</td> </tr> <tr> <td>2</td> <td>1024329970</td> <td>1</td> <td>2018.10...</td> <td>FIA</td> <td>MXP500F-0-5501</td> </tr> </tbody> </table> <p>Example for a SCIEX 5500 series – UHPLC</p>	0	Plate Bar Code	...	Run Time	OP Type	OP	1	1024329965	1	2018.10...	LCMS	MXP500L-0-5511	2	1024329970	1	2018.10...	FIA	MXP500F-0-5501
0	Plate Bar Code	...	Run Time	OP Type	OP															
1	1024329965	1	2018.10...	LCMS	MXP500L-0-5511															
2	1024329970	1	2018.10...	FIA	MXP500F-0-5501															
4	Generate the pipetting layout and autosampler worklist by selecting “Export Worklist for MS” in Met/DQ™.	 <p>Export Worklist for MS ▾</p>																		
5	<u>Export Worklist for MS window:</u> <ul style="list-style-type: none"> • select the option “Order by Acquisition method”. 	 <p>Order by</p> <p><input type="radio"/> Sample</p> <p><input checked="" type="radio"/> Acquisition method</p>																		

7.4.6 Preparing the Kit Plate



1. Make sure that all samples and the kit plate layouts are registered in Met/DQ™ and that the Met/DQ™ acquisition batch file (.csv) for the Analyst® software was generated.
2. Find the kit plate in the kit box. Remove the plastic bag and follow the step-by-step instructions below to prepare the assay.
3. Follow the lab safety protocol while preparing the Kit. Use a fume hood and gloves.



Analyzing QC level 2 in replicates of five (equally distributed over the plate). This guarantees higher quality data when normalizing data across several plates (see Appendix "Data Normalization" in the Met/DQ user manual). For more information refer to the [EMEA guidelines for bioanalytical method validation](#) (European Medicines Agency, 2011).



If samples from a different matrix than plasma are analysed, use a QC of the corresponding matrix. Run this QC in replicates of five.

Note: if no plasma sample are used, Biocrates QCs may not be analysed in replicates.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Cal5										
B	Zero	Cal6			QC2					QC2		
C	Zero	Cal7										
D	Zero	QC1										
E	Cal1	QC2										
F	Cal2	QC3					QC2					
G	Cal3											
H	Cal4											QC2

Step	Instructions	
1	Remove the plastic lid of the MxP® 500 plate.	
2	<p><u>Pipette 10 µL of each sample (zero samples, calibration standards, QCs and experimental samples):</u></p> <p>Use a single-channel pipette to pipette 10 µL onto the center of each filter. Gently touch the filter inserts with the pipette tip while pipetting the samples. Do not pipette on the walls of the wells and avoid cross-contamination. Use a fresh tip for each sample.</p>	
	Well A1 → <i>blank</i> : do not pipette anything	Blank
	Well B1 → <i>zero</i> : pipette 10 µL of PBS	Zero
	Well C1 → <i>zero</i> : pipette 10 µL of PBS	
	Well D1 → <i>zero</i> : pipette 10 µL of PBS	
	Well E1 → <i>cal1</i> : pipette 10 µL of <i>Calibration Standard level 1</i>	Cal1-7
	Well F1 → <i>cal2</i> : pipette 10 µL of <i>Calibration Standard level 2</i>	
	Well G1 → <i>cal3</i> : pipette 10 µL of <i>Calibration Standard level 3</i>	
	Well H1 → <i>cal4</i> : pipette 10 µL of <i>Calibration Standard level 4</i>	
	Well A2 → <i>cal5</i> : pipette 10 µL of <i>Calibration Standard level 5</i>	
	Well B2 → <i>cal6</i> : pipette 10 µL of <i>Calibration Standard level 6</i>	
	Well C2 → <i>cal7</i> : pipette 10 µL of <i>Calibration Standard level 7</i>	
	Well D2 → <i>QC1</i> : pipette 10 µL of <i>Quality Control level 1</i>	QC1
	Well E2 → <i>QC2</i> : pipette 10 µL of <i>Quality Control level 2</i>	QC2
	Well F2 → <i>QC3</i> : pipette 10 µL of <i>Quality Control level 3</i>	QC3
	Well G2 – H12 → pipette 10 µL according to the MetIDQ™ plate layout	Samples
		QC2

Step	Instructions
3	Dry down the samples for 30 min at room temperature under nitrogen flow.
	<p><u>Dry the samples:</u> Use a nitrogen evaporator <u>or</u> pressure manifold.</p> <p>Nitrogen Evaporator:</p> <ul style="list-style-type: none"> • Make sure the evaporator needles are approximately 5 mm above the filter inserts. • Adjust the pressure at the nitrogen pressure reduction valve to about 3-4 bar and check that there is a moderate flow of nitrogen at the end of the evaporator needles. <p>Pressure Manifold:</p> <ul style="list-style-type: none"> • Refer to the “Instructions using pressure manifolds for 96-well plates with Biocrates Kits” on the USB stick located in the folder “Instructions”.
4	Prepare the <i>PITC Derivatization Solution</i> , see section 7.4.2 (page 66).
	<p>Use the <i>PITC Derivatization Solution</i> shortly after adding PITC to the Pre-Mix.</p>
5	Pipette 50 µL of the <i>PITC Derivatization Solution</i> (see 7.4.2) to each well (incl. the <i>blank</i> , well A1). Pipette directly on the center of the filter. Do not pipette on the walls of the wells. We recommend the use of an Eppendorf Multipette® adjusted to <u>medium</u> dispensing speed.
6	Cover the kit plate with the plastic lid and incubate at room temperature for 1 h.
7	Remove the plastic lid. Dry the filters of the kit plate with a nitrogen evaporator <u>or</u> pressure manifold for 1 h.
	Make sure the kit plate is completely dried. Ineffective evaporation of PITC and pyridine will impair the kit performance.
8	Add 300 µL of <i>Extraction Solvent</i> (see 7.4.3) to each well. Use an Eppendorf Multipette® (re-peater) adjusted to <u>low</u> dispensing speed or an 8-channel pipette
9	Cover the kit plate with the plastic lid. Shake the kit plate at room temperature using an Eppendorf® ThermoMixer® or MixMate® at 450 rpm for 30 min.

Step	Instructions
	Be careful when shaking the Kit plate with another shaker as specified in step 9. Due to the risk of cross-contamination, adjust the speed of the mixer carefully. Make sure there is no spill over.
10	Centrifuge the kit plate for 2 min at 500 × g (make sure the centrifuge is balanced) or use a pressure manifold (see “Instructions using pressure manifolds for 96-well plates with Biocrates Kits” on the USB stick located in the folder “Instructions”).
	After the elution, check that the fill levels are similar in all wells of the capture plate If not, apply higher g-force or higher pressure, respectively.
11	Carefully remove the tapes from the sides of the kit plate. Separate the lower capture plate (containing the sample extracts, labeled “Use for FIA”) from the upper filter plate. Take care that spills and splashes are avoided during this process.
	In case of any delays, seal the capture plate with a silicon mat and store it at 4 °C before continuing with the next steps. The plate can be stored up to two days at 4 °C. Do not store the plate below 0 °C!
	The following preparation steps 12 – 15 should be performed immediately.

Step	Instructions
5500 series	
	<p>For this step you need:</p> <ul style="list-style-type: none"> • Capture plate labeled "Use for FIA" from step 11 • Empty 96 deep well plate labeled "Use for LC" (provided with kit box) • Empty 96 deep well plate labeled "Use for FIA dilution - If required" (provided with kit box) • 2 silicone mats (provided with kit box)
12a	<p>Split extracts:</p> <ol style="list-style-type: none"> 1. Remove 150 µL from each well of the capture plate (labeled "Use for FIA") and transfer volumes to the empty 96 deep well plate labeled "Use for LC". 2. Remove 10 µL from each well of the capture plate (labeled "Use for FIA") and transfer volumes to the empty 96 deep well plate labeled "Use for FIA dilution - If required".
	<p>Make sure you transfer each sample exactly to the appropriate well position of the empty deep well plate. We recommend the use of a multichannel pipette. Condition pipette tips: aspirate and dispense extracts 3 times before transferring!</p>
	<p>Run the LC plate (labeled "Use for LC") first, for reason of analyte stability. Run the LC plate the next day after preparation at the latest.</p>
	<p>The LC and FIA analyses are performed using two separate plates at different concentrations.</p>
13a	<p>Dilute extracts for LC part: Use the plate labeled "Use for LC". Add 150 µL of water to each well. Seal the plate with a silicone mat. Shake for 10 min at 600 rpm.</p>
14a	<p>Dilute extracts for FIA part: Use the plate labeled "Use for FIA dilution - If required". Add 490 µL of FIA solvent to each well. Seal the plate with a silicone mat. Shake for 10 min at 600 rpm.</p>
15a	<p>Both plates, labeled "Use for LC" and "Use for FIA dilution - If required", are now ready for LC-MS and FIA-MS analysis, respectively. Place the sealed plates in the autosampler at 10 °C or store at 4 °C.</p>
	<p>In case of any delays store the plates at 4 °C. The autosampler stability is:</p> <ul style="list-style-type: none"> • LC part: 2 days • FIA part: 7 days. <i>In case of extract evaporation, fill up the wells to the original volume with methanol and shake the plate.</i> • Never store the LC plate or the FIA plate below 0 °C!
16a	<p>Continue with section 8, page 75.</p>

8 Processing the Kit Plate with the Mass Spectrometer



For MS data acquisition two options are available using the Analyst® software:

- one file per injection is created, only .wiff files – **recommended**
- two files per injection are created, .wiff and .scan files

Check the Analyst® settings according to section 11.3.

Before starting the measurements, set up the Analyst® acquisition methods as described below. Make sure that all parameters (see section 4) were added to the methods and that the solvents match the method settings (e.g. solvent line A1 or A2). Make sure that the mass spectrometer meets all manufacturer specifications and has been **tuned and calibrated for positive and negative ion mode**. Take care that the 96 deep well plates (labeled “Use for LC” and “Use for FIA dilution - If required”) are sealed with silicone mats. Place these plates in the correct autosampler racks. This process using the Analyst® software is shown in a video tutorial.



The mass spectrometer must meet all manufacturer specifications and must be recently **tuned and calibrated for positive and negative ion mode**.

The autosampler must be suitable for 1 mL 96 deep well plates.



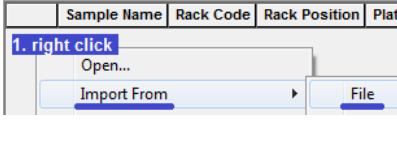
[9A MS Measurement: Batch File/Acquisition Method Import for AB Sciex MS Instruments](#)



Perform the SST according to section 6 System Suitability Test (SST) before sample analysis using the Kit in a mass spectrometer. If the SST fails, do not start sample analysis and perform troubleshooting! If required, contact the SCIEX or Biocrates® customer support.

8.1 LC Part

We recommend running the LC-MS assay first. Some LC analytes, e.g. biogenic amines, are less stable in the autosampler than the analytes of the FIA part. For the LC-MS data, the Met/DQ™ software is used for data processing and the quantitation process.

ESI probe position:	
	5500 series
i	x-axis 5 y-axis 5
Step	Instructions
1	Place the LC kit plate, labeled “Use for LC”, in the autosampler.
2	Start Analyst® and build a new acquisition batch: File > New > Acquisition Batch.
	
3	Create a new acquisition batch: Import the .csv file (created by Met/DQ™, see 7.4.5, page 69) into Analyst® before instrumental analysis. Follow the steps below: <ol style="list-style-type: none"> 1. Right click on the New Batch window. 2. Select Import From > File. 3. Change the file type to .csv. 4. Select the Met/DQ™ worklist export (.csv file) and click Open.
	
4	Check that the acquisition methods <u>MxP500L-LC1_5511.dam</u> and <u>MxP500L-LC2_5511.dam</u> are selected.
i	Two “Sets” are used to perform injections with the acquisition methods LC1 and LC2. 

Step	Instructions
5	Define the correct <i>Rack Code</i> , <i>Rack Position</i> , <i>Plate Code</i> , <i>Plate Position</i> , and <i>Vial Position</i> .
	Do not rename or alter the samples, data files, or acquisition methods. Otherwise the data files will not be compatible with MetIDQ™.
6	Submit the sequence and equilibrate the LC-MS system. <input type="button" value="Submit"/>  
	Before injecting, wait until all LC-MS parameters are stable. The blank sample will be injected three times to condition the system. Double-check that two vials for “water addition” are in the autosampler, see section 11.1.4.
7	Run the batch. To ensure that the assay is running properly, monitor the first injections including data acquisition. 

8.2 FIA Part

ESI probe position:



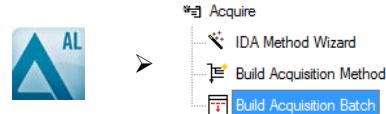
5500 series	
x-axis	5
y-axis	5

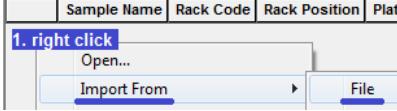
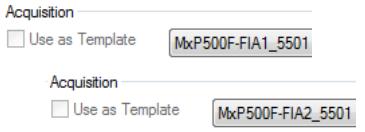
The FIA part is more sensitive to contaminations in the autosampler compared to the LC part, as hundreds of lipids are analyzed. In our experience, autosamplers are often contaminated with lipids. Make sure that all system parts are clean and the noise level is low. If necessary, clean autosampler parts such as needle, needle seat, sample loop, and switching valves.



Perform the SST according to section 6.4.3 before processing the kit in a mass spectrometer. If the SST fails, do not start analysis and perform troubleshooting! If required, contact the SCIEX or Biocrates® customer support.

Step	Instructions
1	Remove the column and connect the autosampler directly to the ion source. Alternatively, a bypass or separate column line (without a column installed) can be used. In this case, make sure to use as few connecting parts as possible to minimize dead volume and obtain the best FIA results possible.
2	Place the FIA kit plate in the autosampler, labeled "Use for FIA dilution-If required".
3	Before the first injection flush all lines with FIA solvent, e.g. flow rate 0.2 mL/min for 10 min.
	Activate a MS method while applying a flow through the ion source.
4	Start Analyst® and build a new acquisition batch: File > New > Acquisition Batch.



Step	Instructions
5	<p>Create a new acquisition batch:</p> <p>Import the .csv file (created by Met/DQ™, see 7.4.5, page 69) into Analyst® before instrumental analysis. Follow the steps below:</p> <ol style="list-style-type: none"> 1. Right click on the New Batch window. 2. Select Import From > File. 3. Change the file type to .csv. 4. Select the Met/DQ™ worklist export (.csv file) and click <i>Open</i>.  <p>File type: Microsoft Text Driver (*.txt; *.csv)</p>
6	<p>Check that the acquisition methods <i>MxP500F-FIA1_5501.dam</i> and <i>MxP500F-FIA2_5501.dam</i> are selected.</p> 
	<p>Two “Sets” are used to perform injections with the acquisition methods FIA1 and FIA2.</p> <p>Set: <input checked="" type="checkbox"/> SET0 <input type="checkbox"/> SET1</p>
7	<p>Define the correct <i>Rack Code</i>, <i>Rack Position</i>, <i>Plate Code</i>, <i>Plate Position</i>, and <i>Vial Position</i>.</p>
	<p>Do not rename or alter the sample names, data files, or acquisition methods. Otherwise the data files will not be compatible with Met/DQ™.</p>
8	<p>Submit the sequence and equilibrate the LC-MS system.</p> <p><input type="button" value="Submit"/> ➤ <input type="button" value="Equilibrate"/></p>
	<p>Before starting an injection, wait until all LC-MS parameters are stable.</p> <p>The blank sample will be injected three times to condition the system.</p>
9	<p>Run the batch.</p> <p>To ensure that the assay is running properly, monitor the first injections including data acquisition.</p> <p></p>

Recommended cleaning of tubing and ESI electrode

The FIA method operates at relatively low flow rates when analyzing biological samples, therefore, deposits can accumulate in the tubing or ESI electrode. It is recommended to integrate routine washing steps to ensure a robust performance of the kit. We suggest the following or similar washing steps.

1. Wash tubing and ESI probe for 60 min using water at a flow rate of 0.5 mL/min.
2. Wash tubing and ESI probe for 60 min using isopropanol at a flow rate of 0.5 mL/min.
3. Wash tubing and ESI probe for 20 min using FIA solvent at a flow rate of 0.5 mL/min.

***Troubleshooting:***

One of the most likely reasons for instabilities in the FIA profile is that there are deposits in the ESI electrode or tubing. It is recommended to monitor the backpressure pressure profile during Kit runs (when the flow rate is at 0.2 mL/min). A significant increase in pressure is an indication for the need to clean or replace the ESI electrode or tubing.

Cleaning of ESI electrode:

Remove the electrode from the source and clean it using an ultrasonic bath. First use 50% isopropanol in water for 15 min and subsequently 100% methanol for 15 min as wash solvents. If samples are routinely analyzed using kits (throughput of several kits per week), it may be required to replace the ESI electrode regularly.

9 Data Processing – LC Part

The quantitation of LC and FIA data is performed by the Met/DQ™ software (for LC data the use of Analyst® is optional). The procedures are described in the Met/DQ™ user manual. The LC data analysis is based on a seven- or one-point calibration and internal standard normalization. Relevant calibration parameters, such as calibration standard concentrations, can be found in the Analytical Specifications. For data analysis, please follow the instructions in this section.

Important: To generate reproducible and longitudinal comparable data, do not change the acquisition method, quantitation method, or software version within one study. For LC data quantitation use either Met/DQ™ or the Analyst® software.

9.1 Quantitation using Met/DQ™

LC data analysis is performed using the Met/DQ™ software. The procedure is described in the Met/DQ™ user manual.



Before starting data analysis, adjust the retention times (RTs) in Met/DQ™. For correct peak identification and integration, refer to section 9.2 *Peak Identification*.



Optionally, LC data analysis can be done using the SCIEX Analyst® software, see next section 9.3. *Optional: Quantitation using Analyst®*.



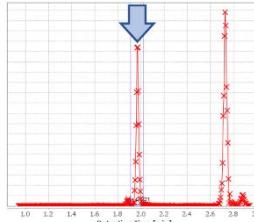
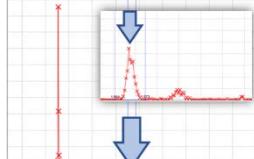
Please refer to the Met/DQ™ Nitrogen user manual section **5.1 LC data – quantitation by Met/DQ™**.

9.2 Peak Identification

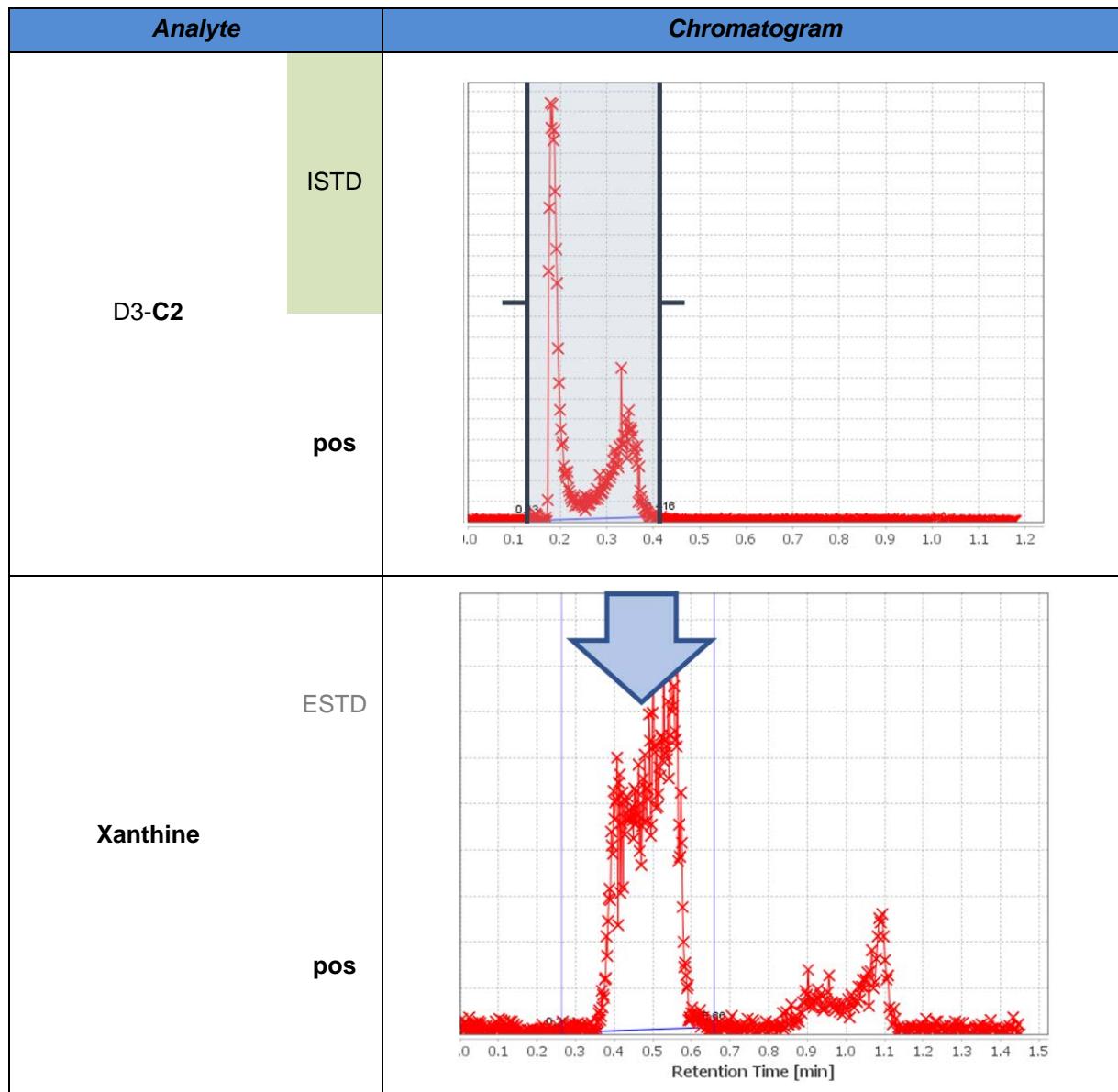
For RT adjustment in Met/DQ™ example Test Sample LC chromatograms are shown, acquired with the acquisition methods

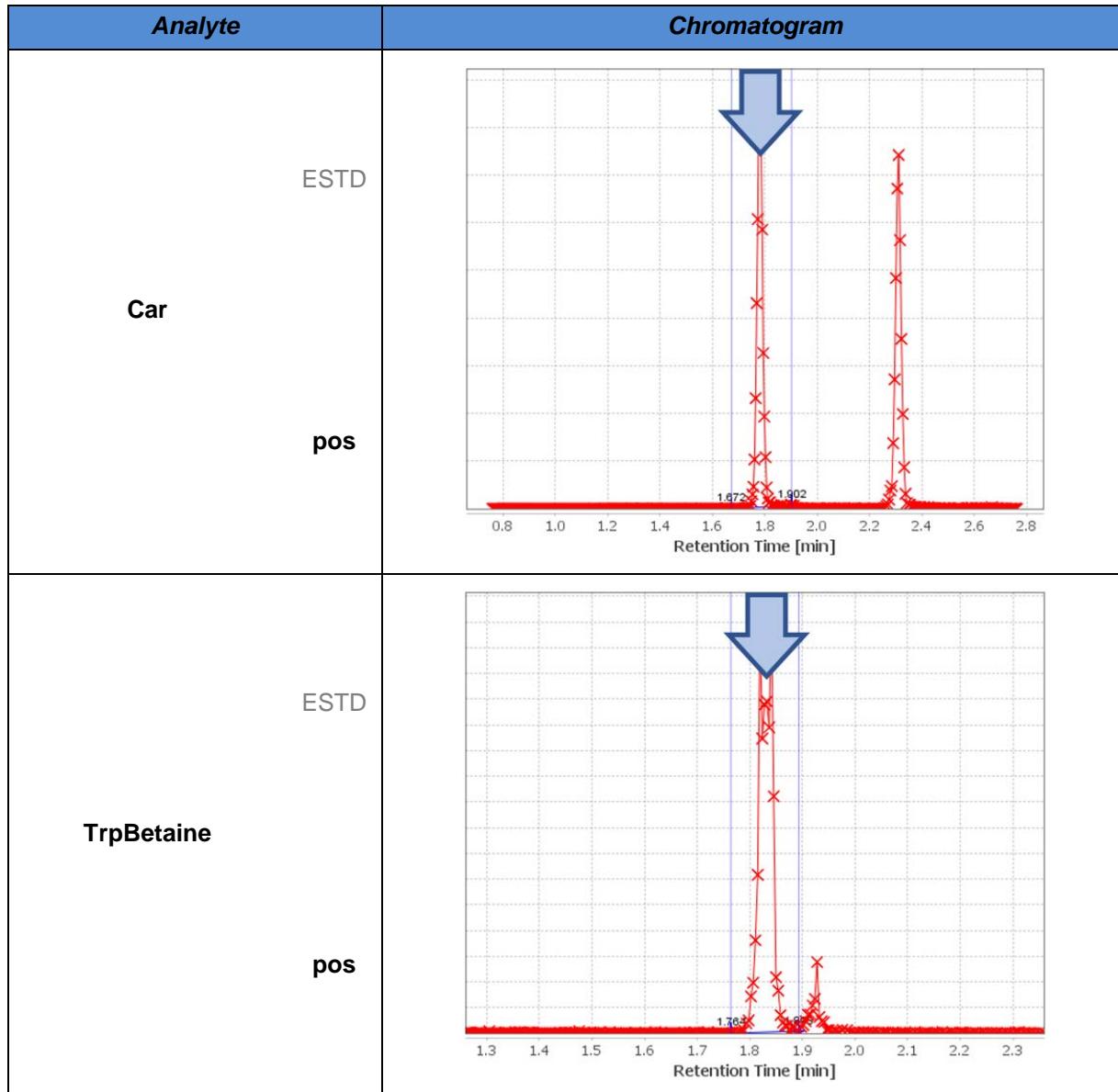
- LC1: [MxP500L-LC1_5511_adjustRT.dam](#)
- LC2: [MxP500L-LC2_5511_adjustRT.dam](#)

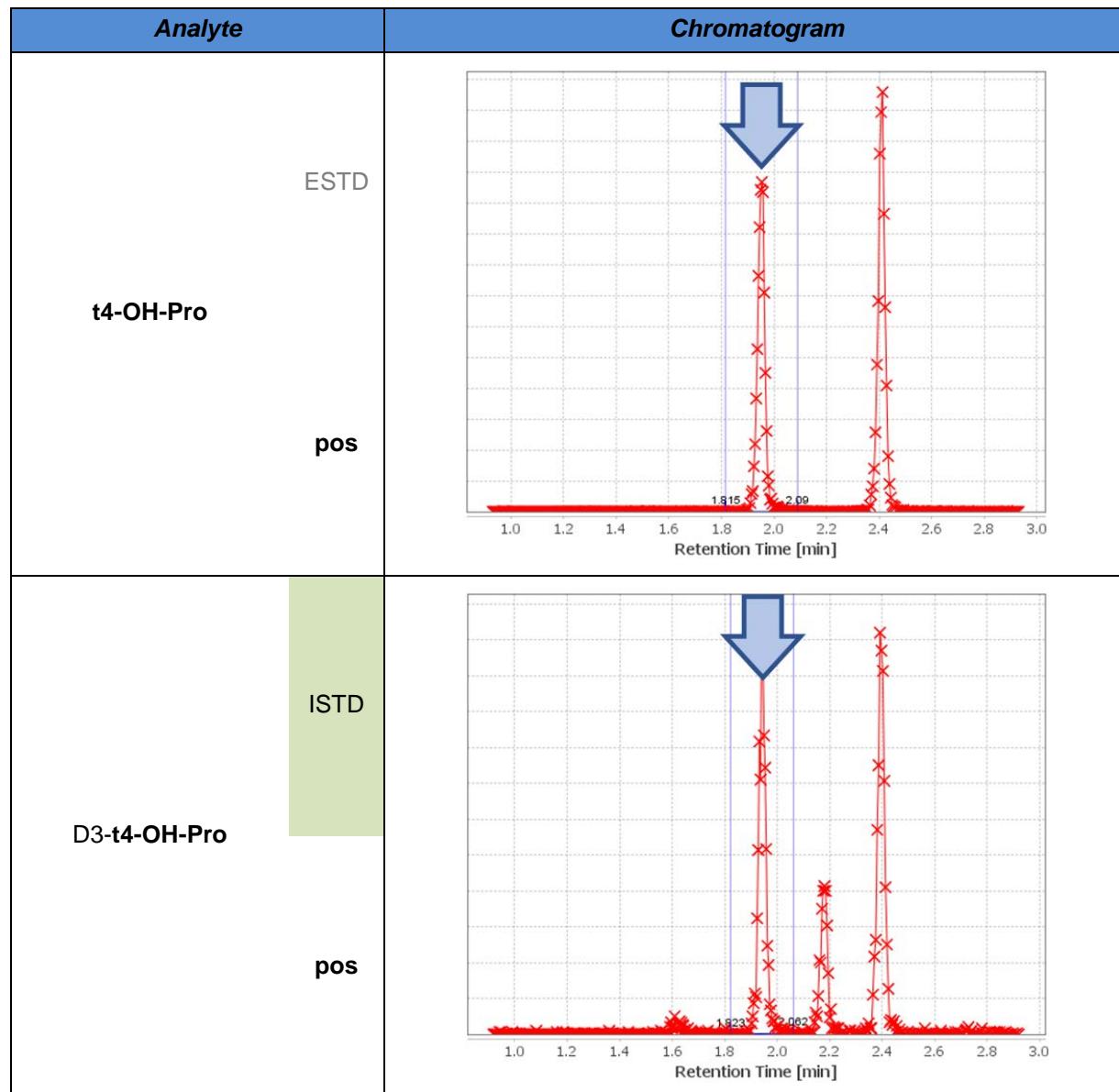
Tags are used to identify external standards (ESTD), internal standars (ISTD), positive mode (**pos**), negative mode (**neg**). An example is shown below.

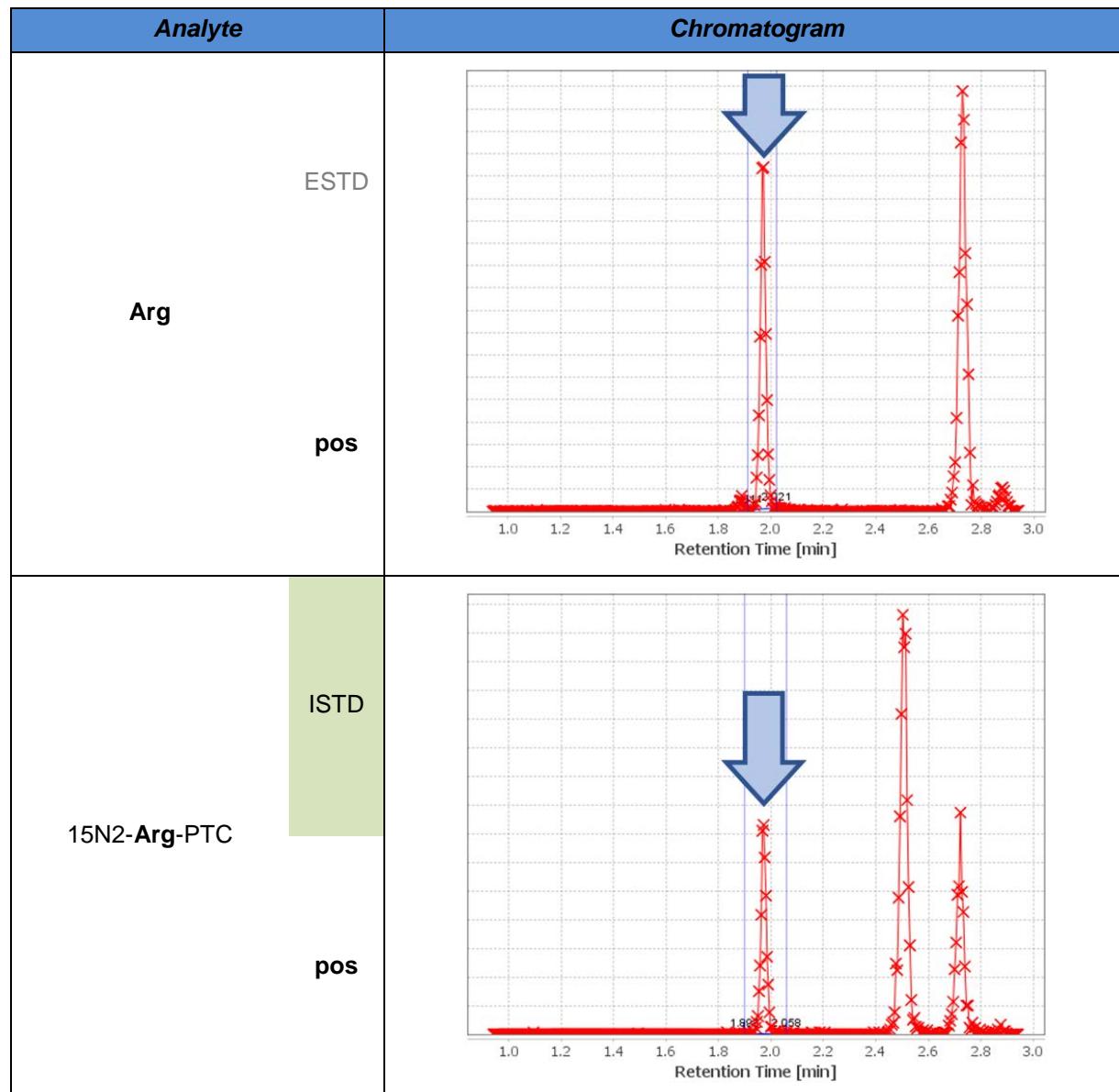
	<i>Analyte</i>	<i>Chromatogram</i>
External standard (pure metabolite)	Arg ESTD	
	pos	
Internal standard	D5-TCA ISTD	
	neg	

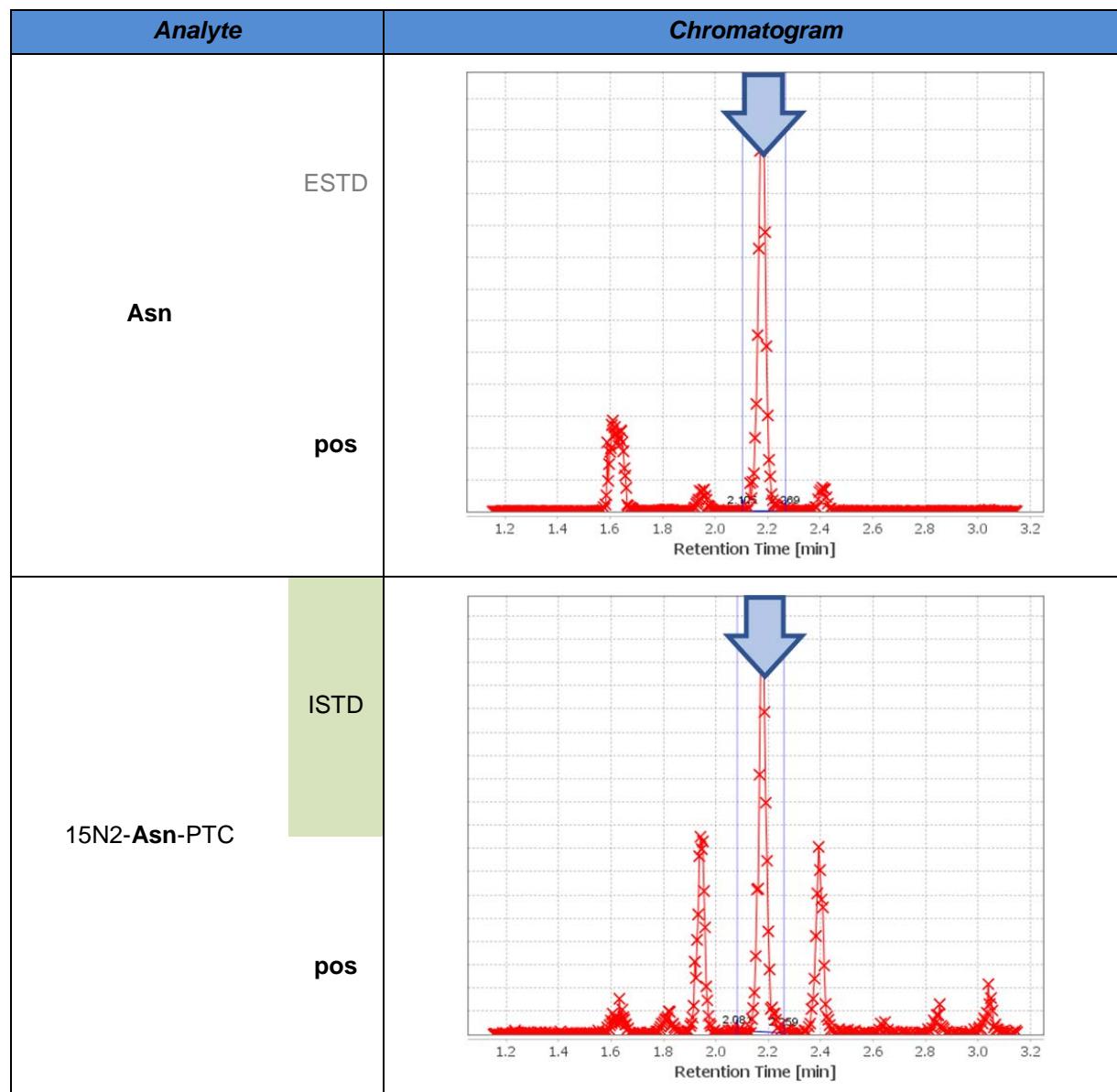
Example chromatograms were acquired with a SCIEX 5500 QTRAP + Agilent® 1290 pump/autosampler. RTs may be different on every LC-MS system.

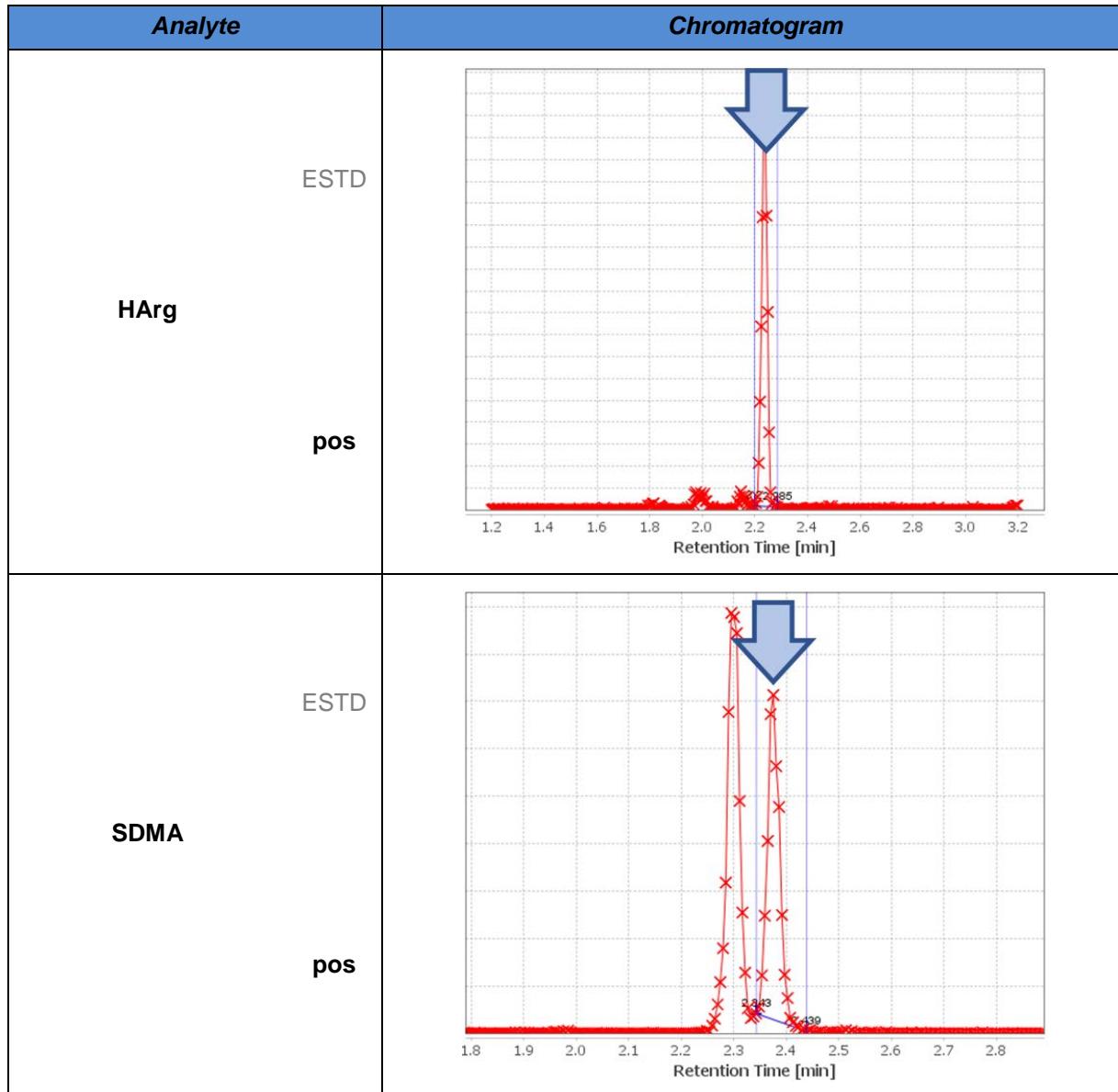


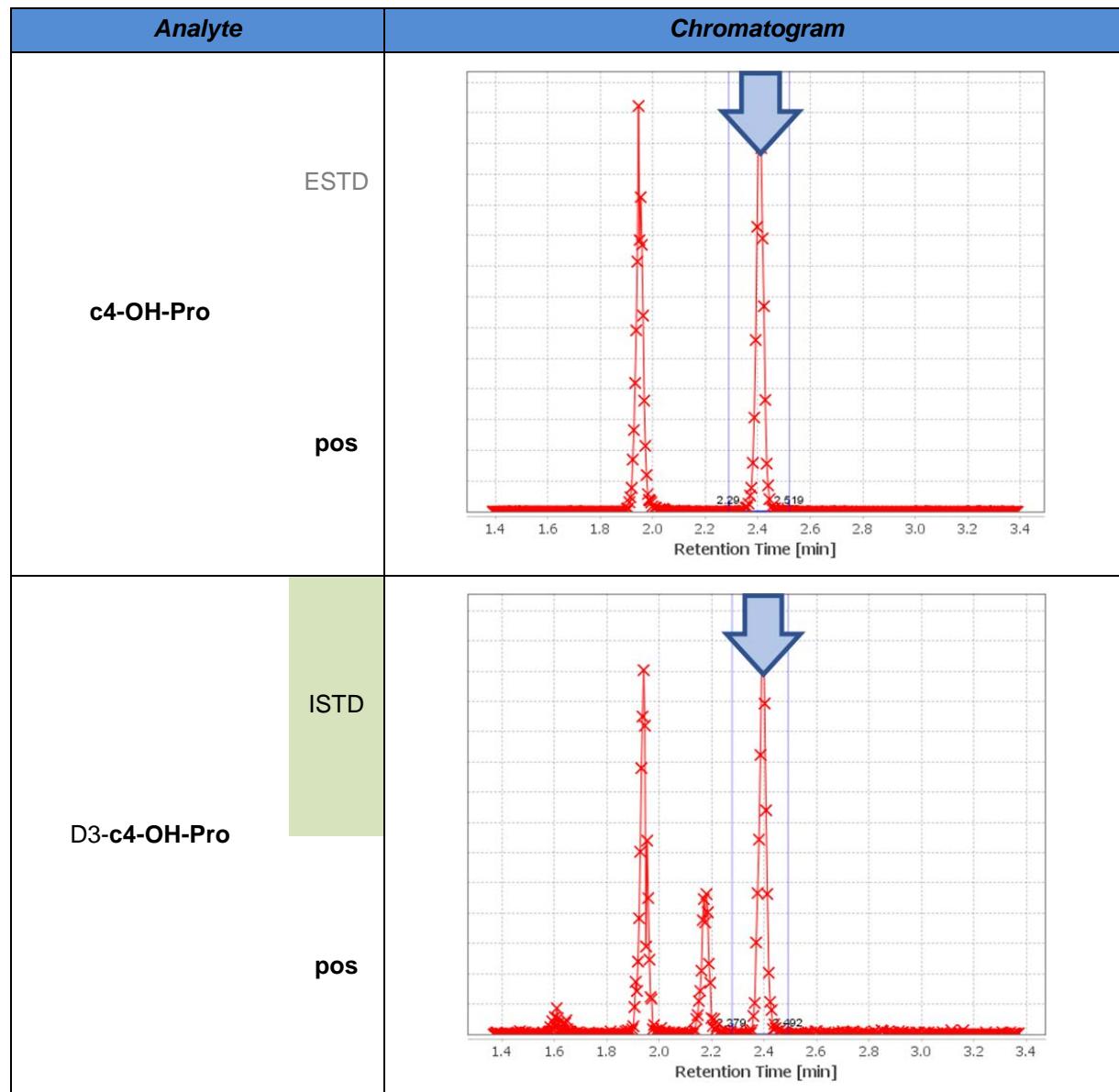


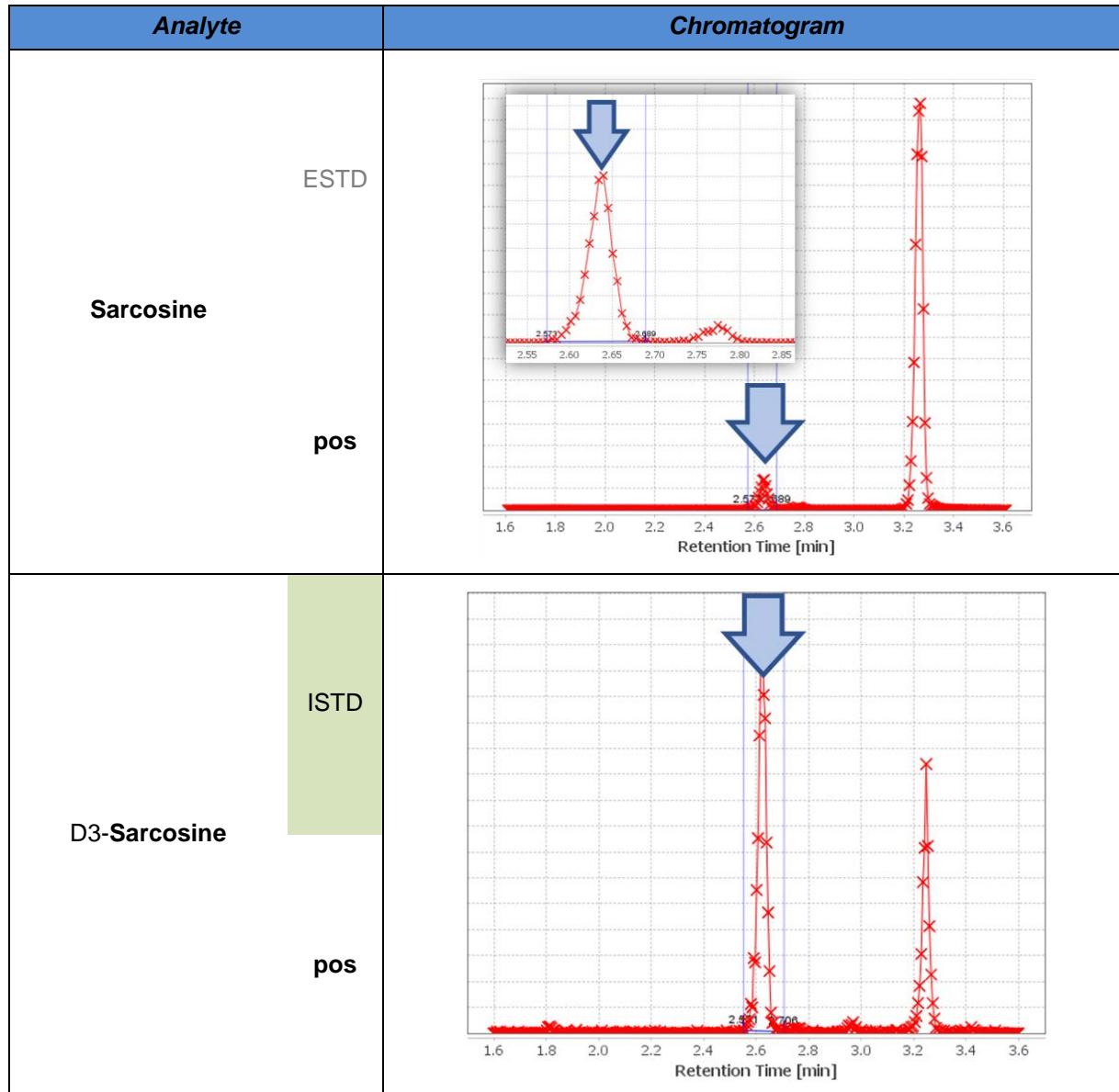


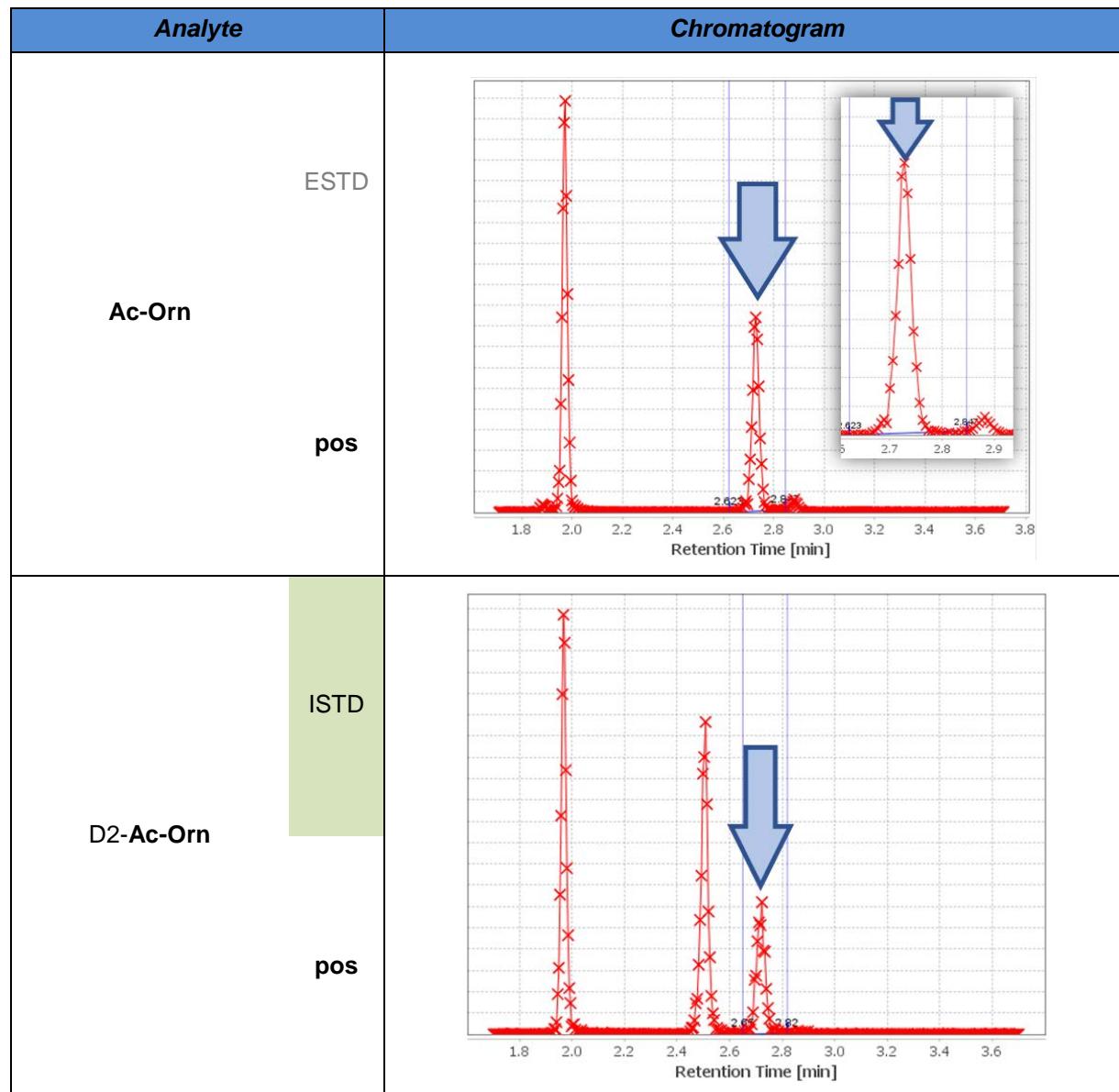


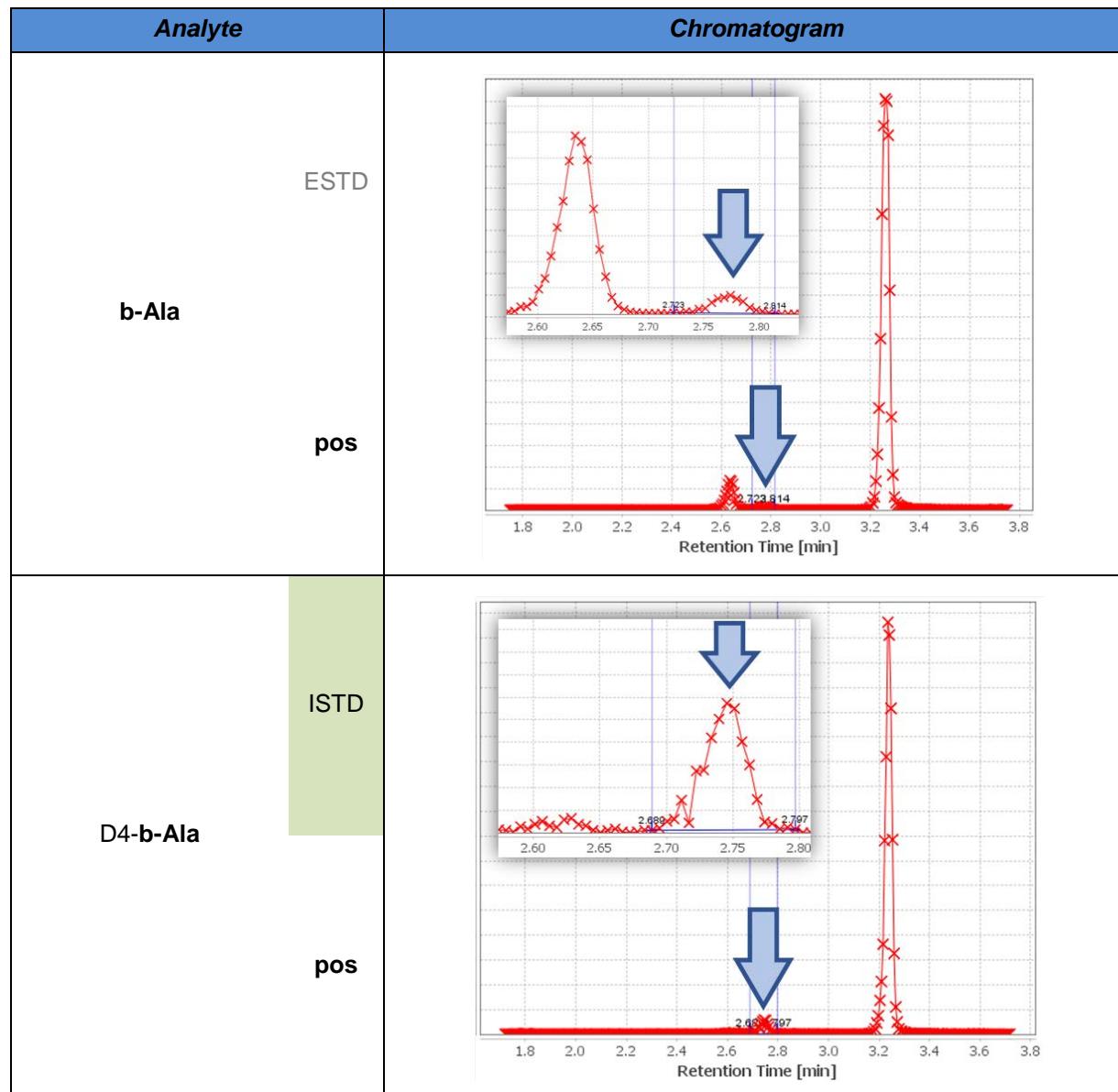


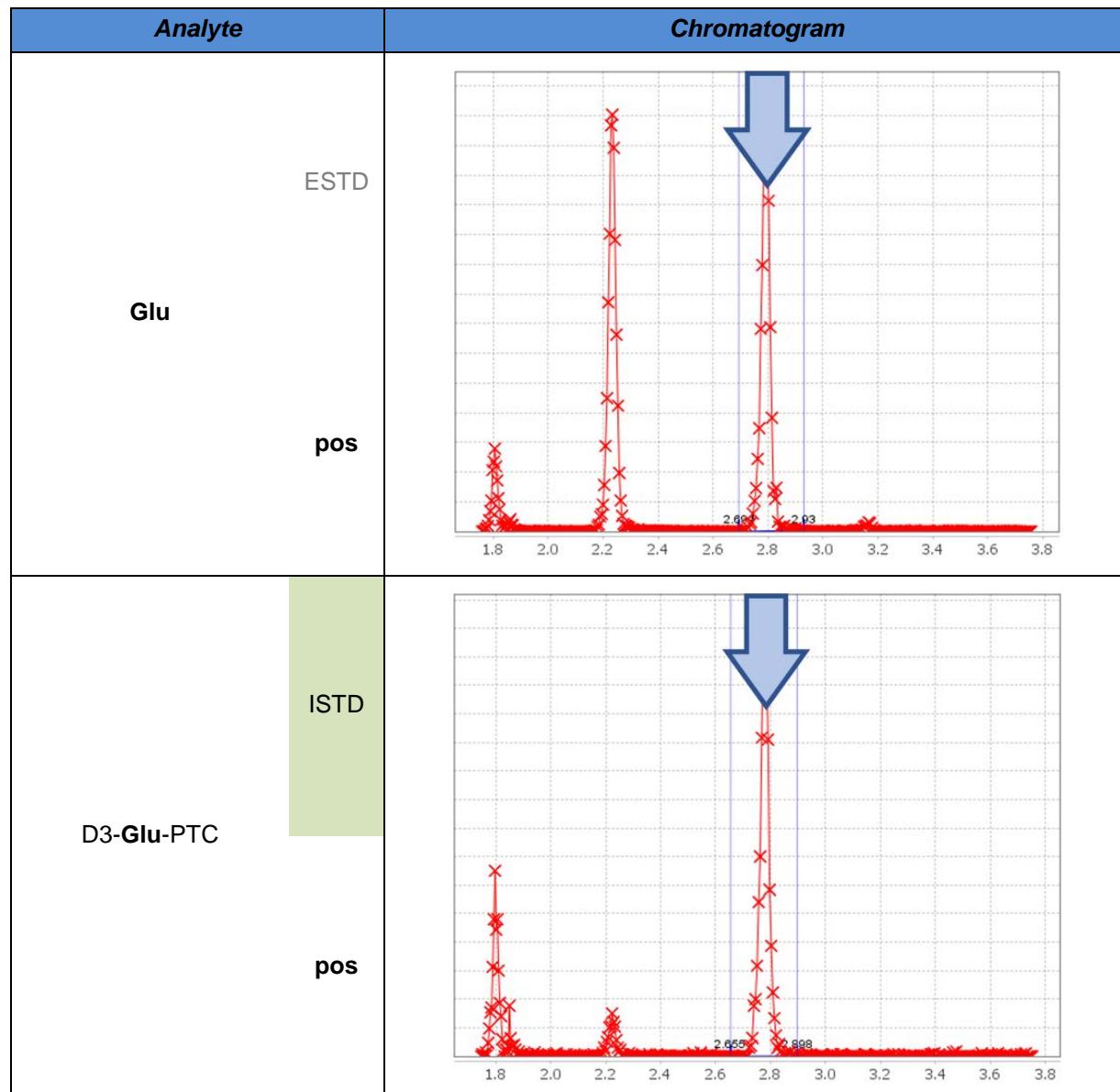


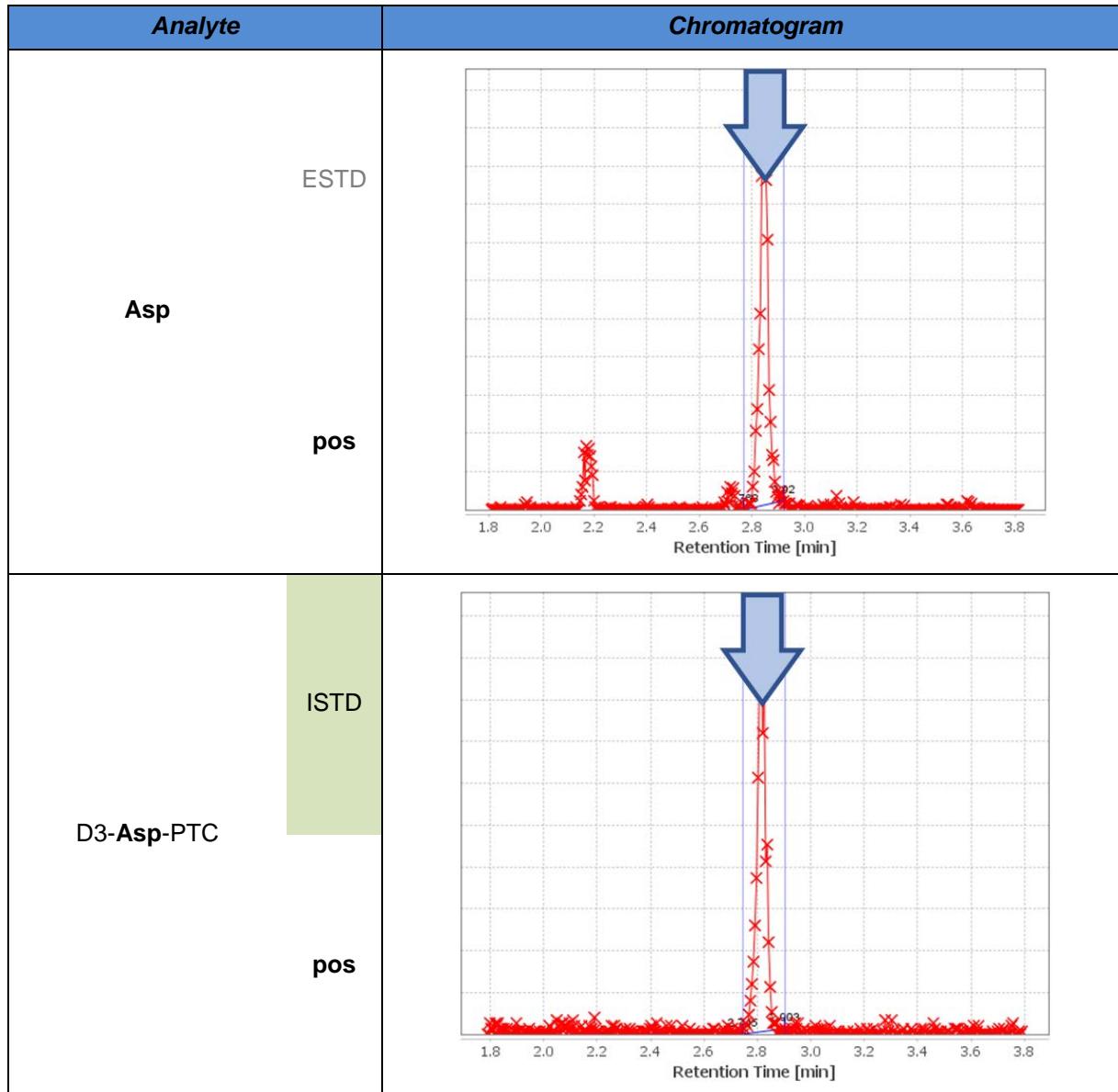


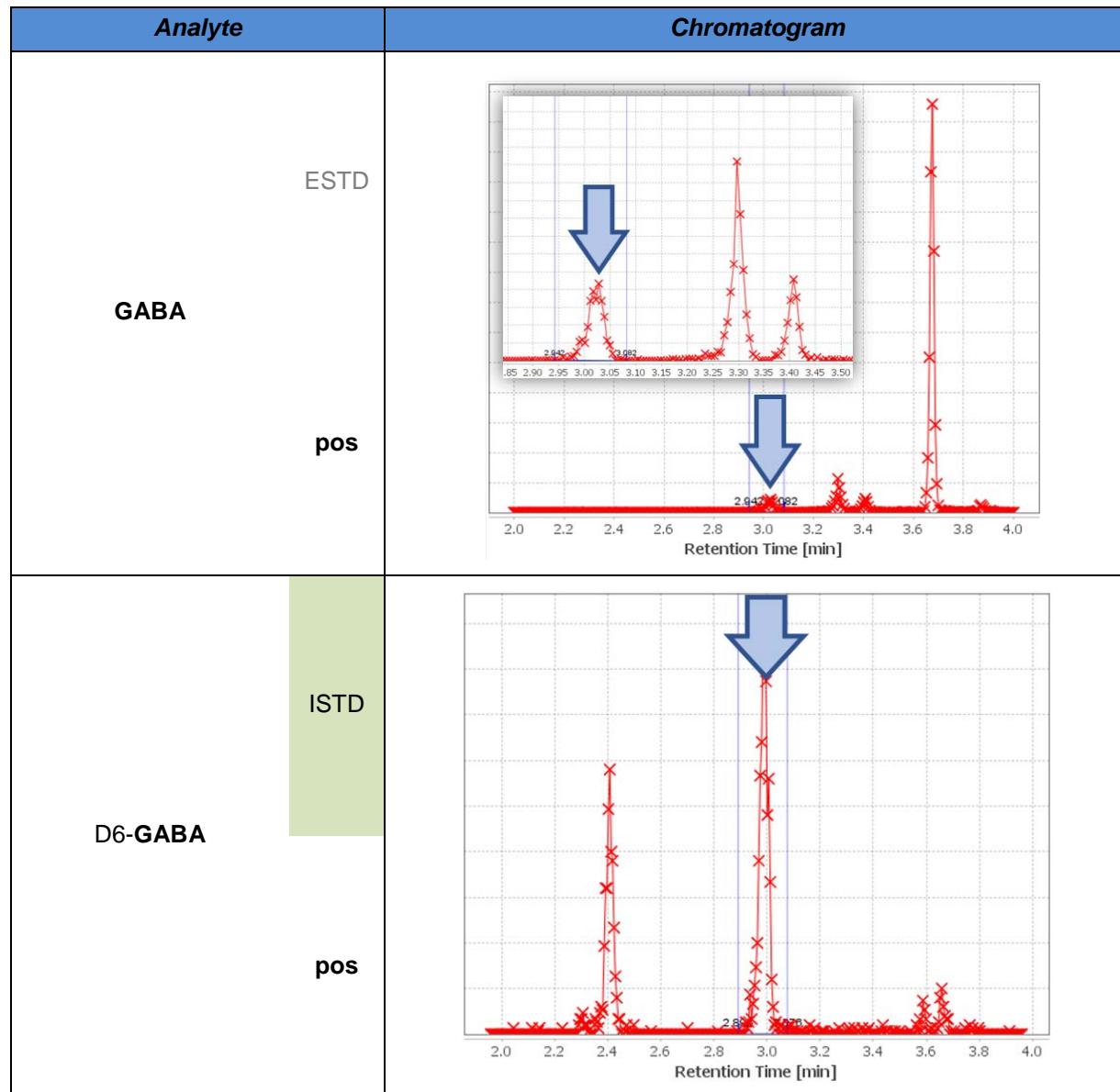


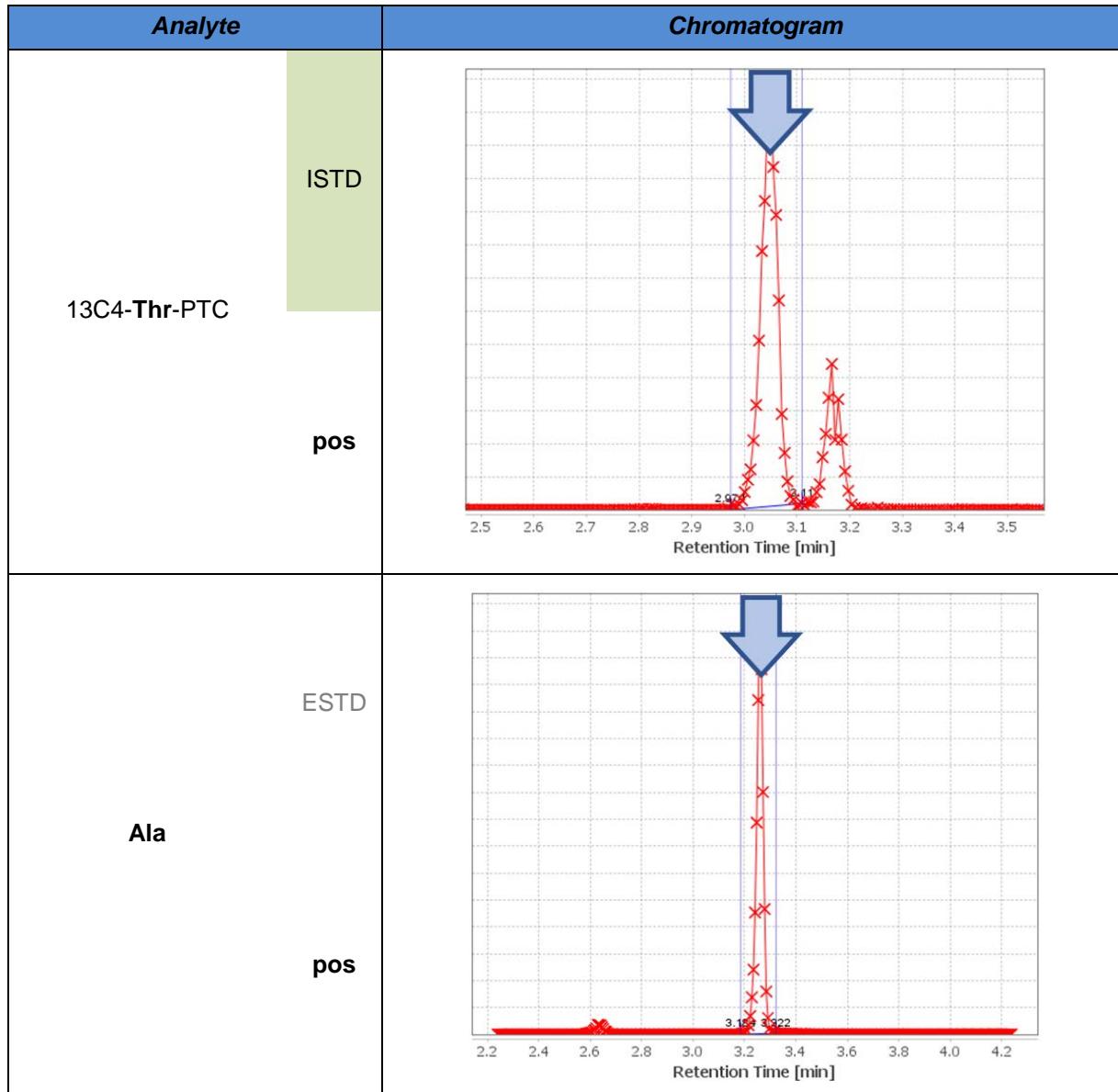


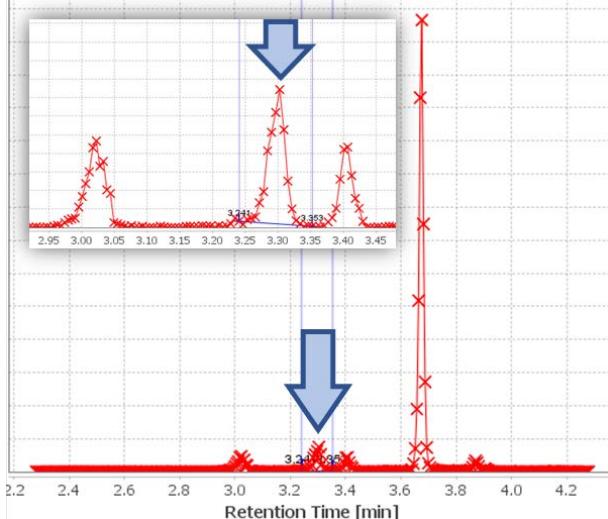
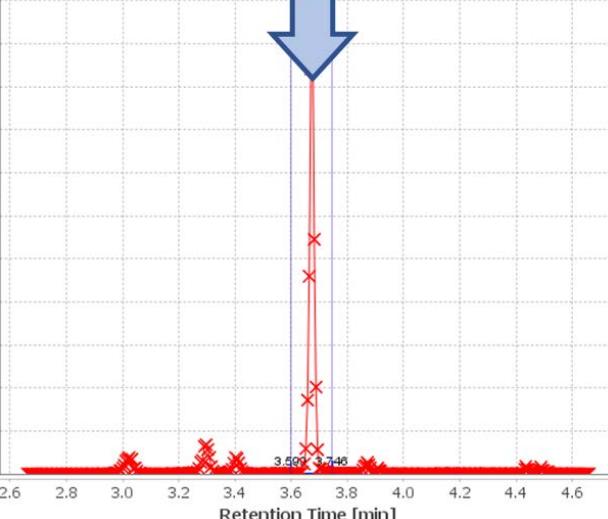


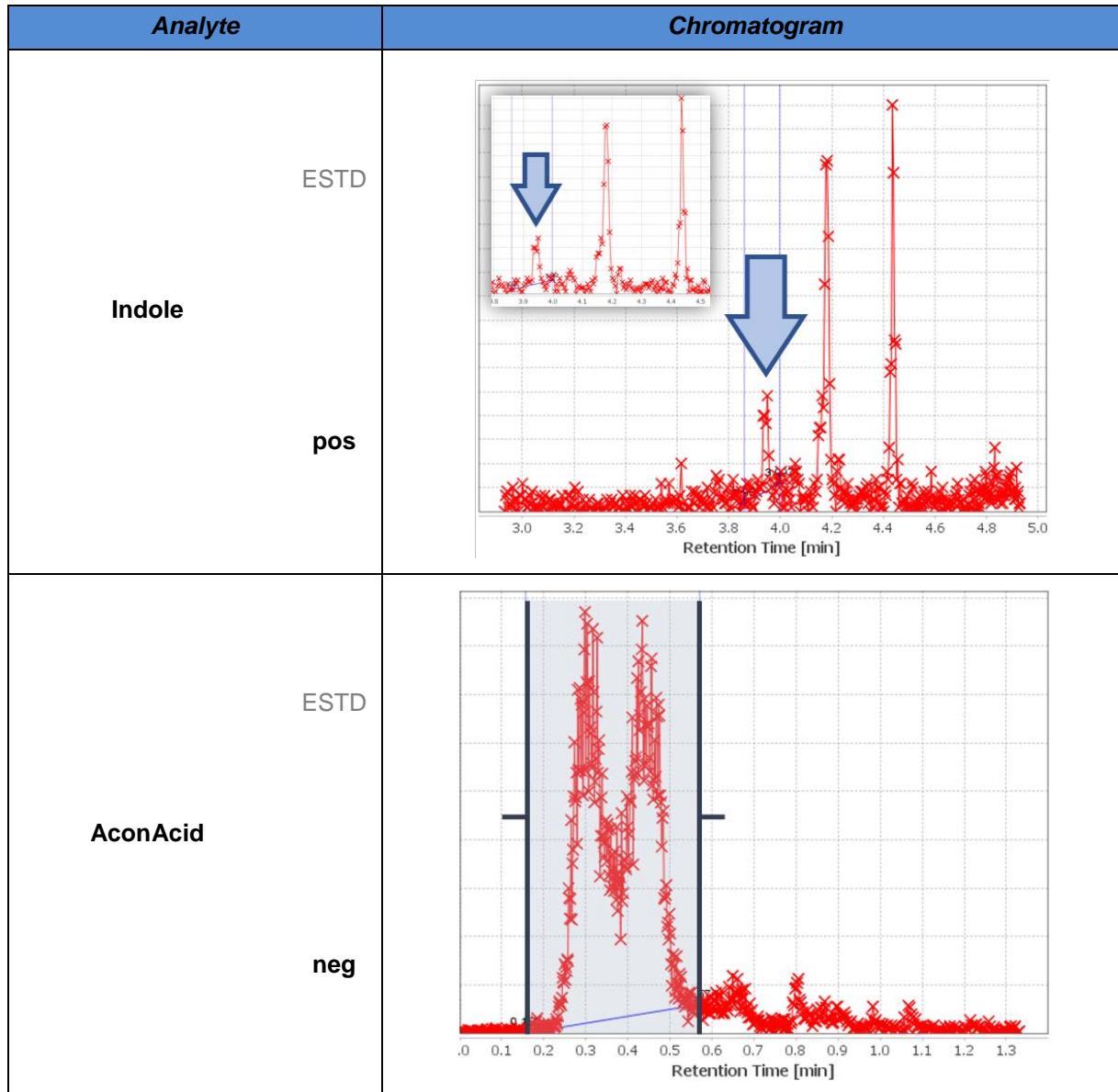


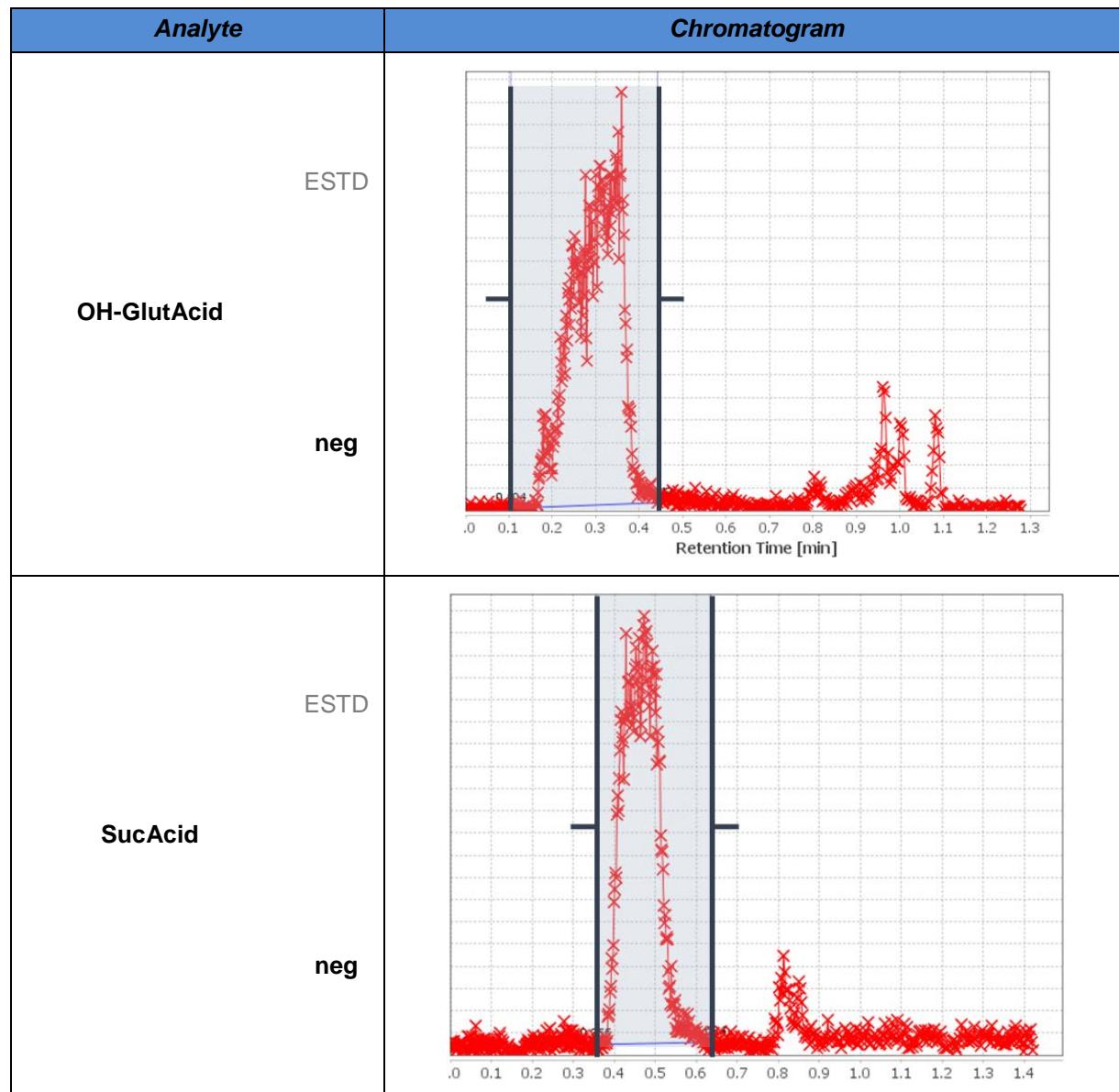


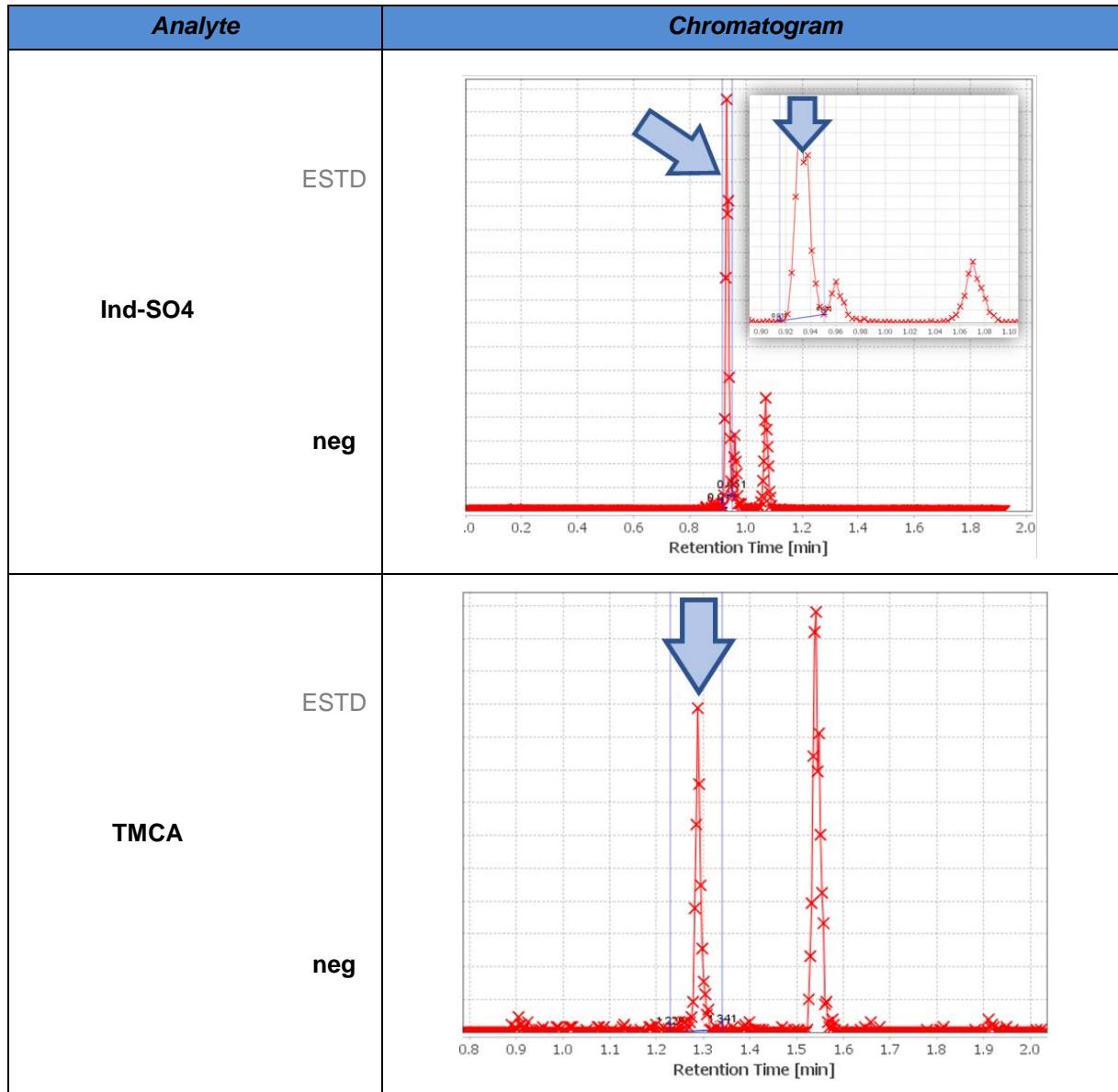


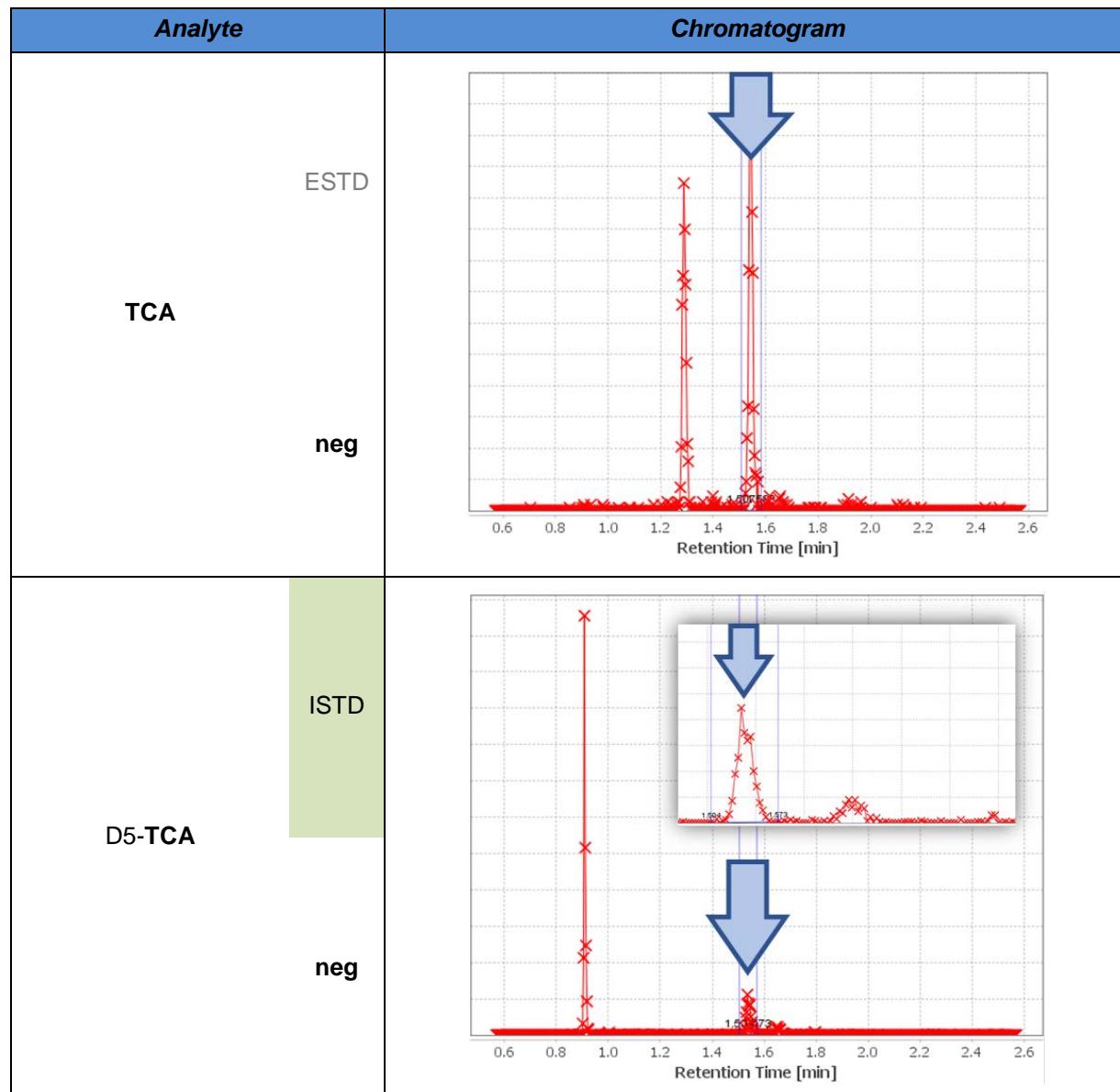


Analyte	Chromatogram
BABA	 <p>ESTD</p> <p>pos</p> <p>Retention Time [min]</p>
AABA	 <p>ESTD</p> <p>pos</p> <p>Retention Time [min]</p>

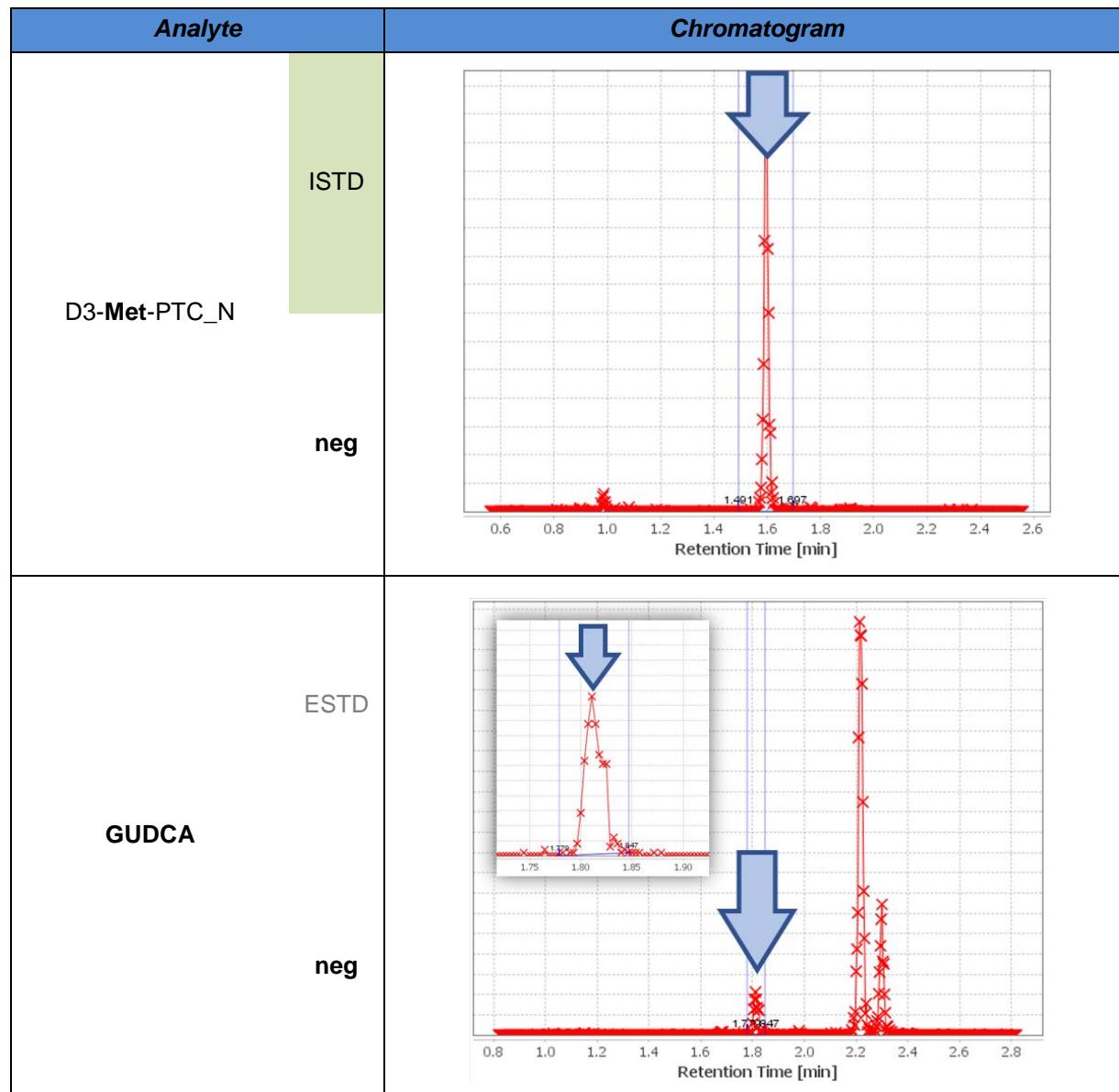


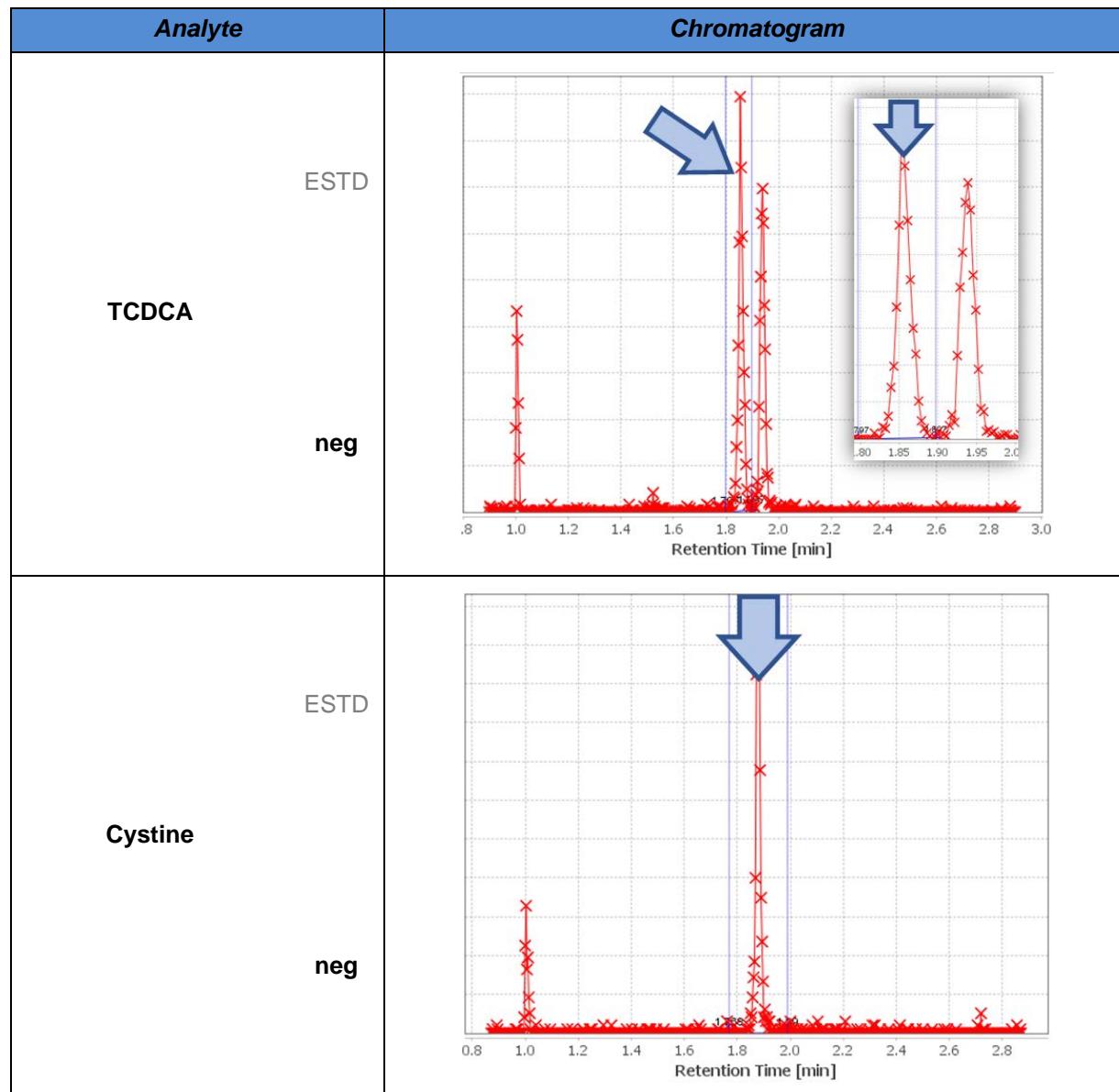


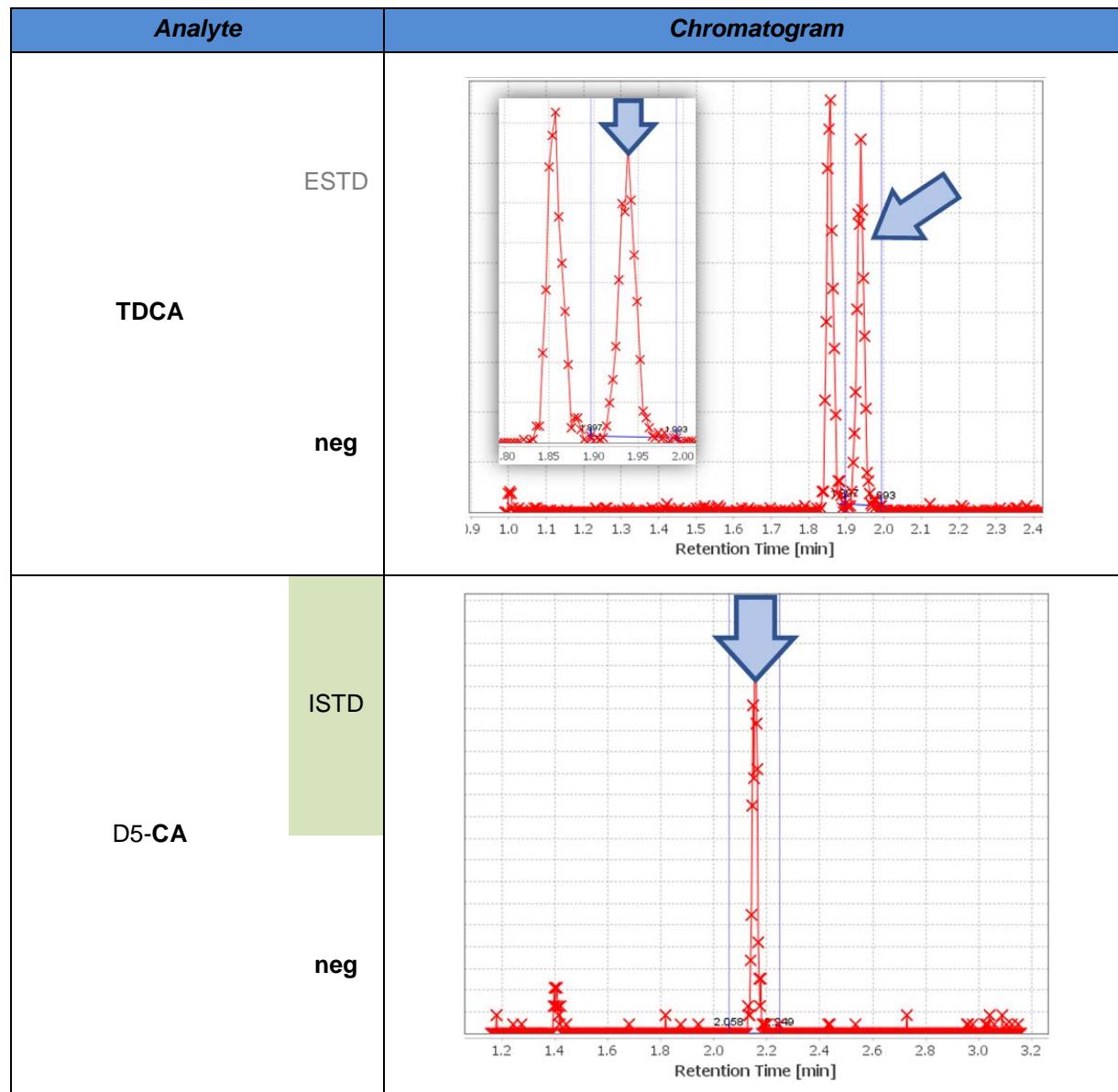


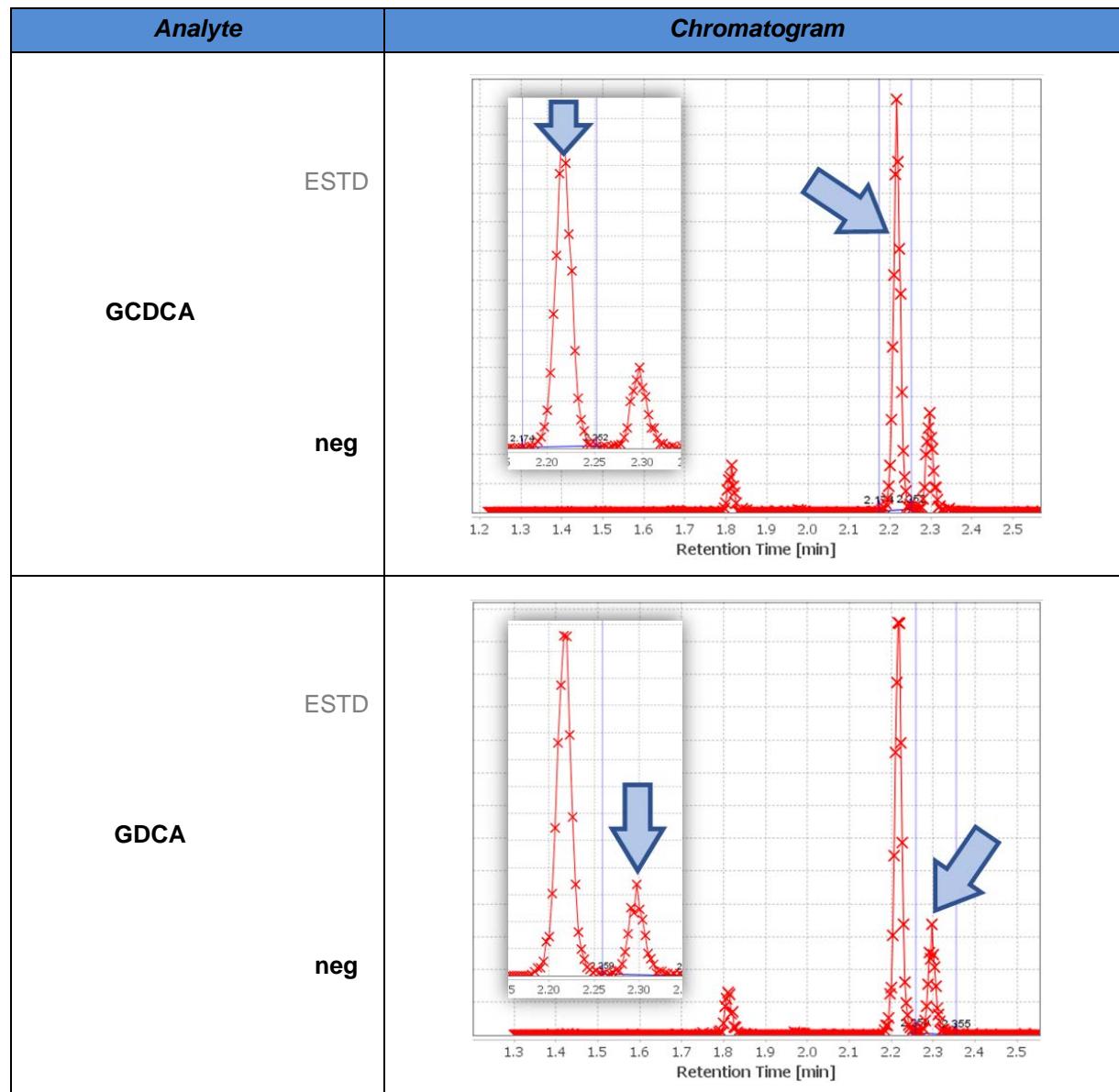


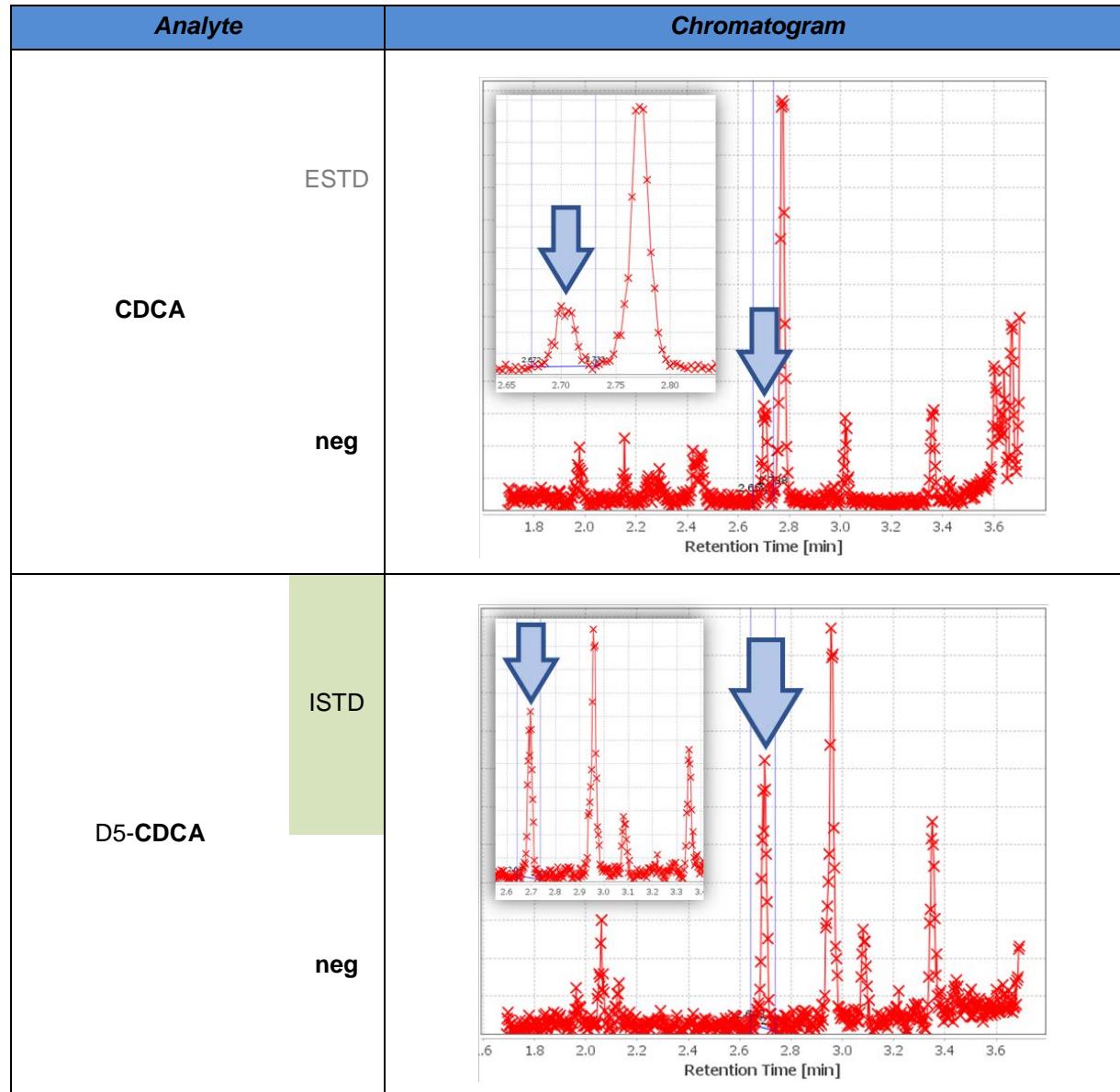
MxP® Quant 500 Kit for SCIEX triple quadrupole instruments

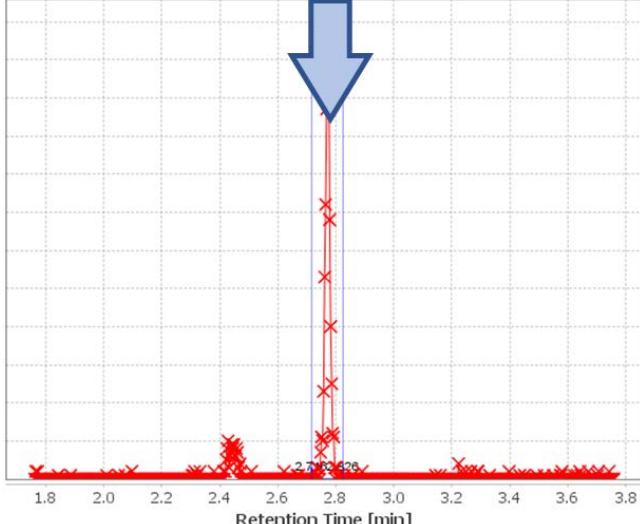
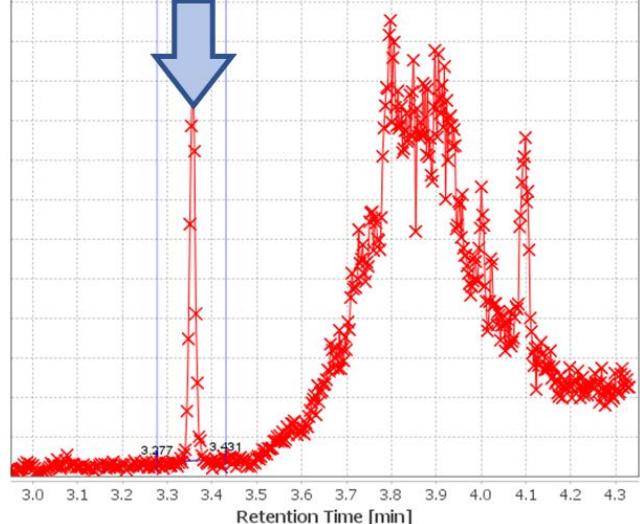


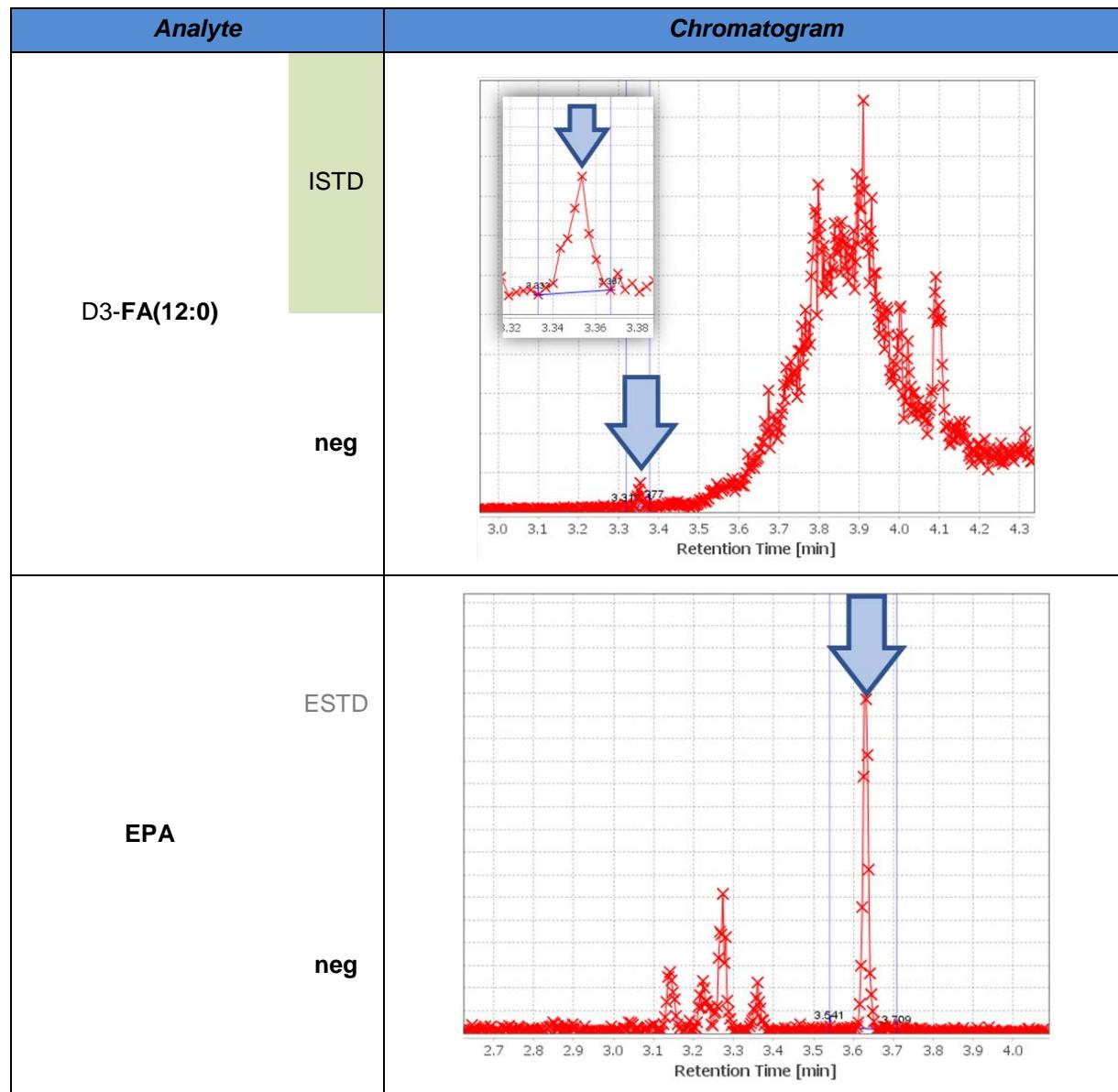


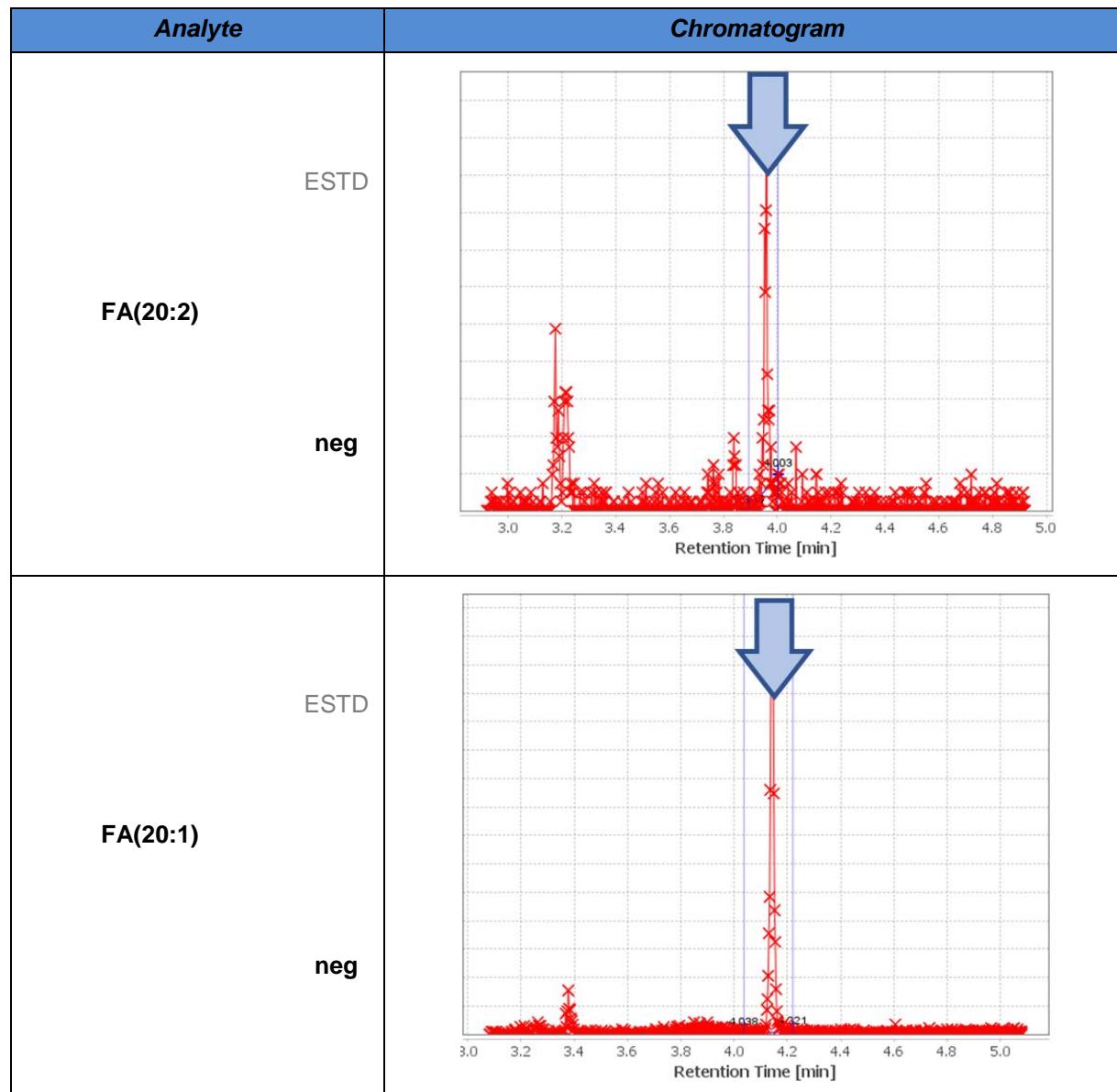






Analyte	Chromatogram
ESTD DCA neg	 <p>Chromatogram showing a single sharp peak at 2.7 minutes. The x-axis is labeled "Retention Time [min]" from 1.8 to 3.8. A blue arrow points to the peak at 2.7 minutes. Red 'x' marks are scattered across the baseline.</p>
ESTD FA(12:0) neg	 <p>Chromatogram showing a complex multi-peak pattern starting around 3.4 minutes and peaking between 3.8 and 4.0 minutes. The x-axis is labeled "Retention Time [min]" from 3.0 to 4.3. A blue arrow points to the initial rise at 3.4 minutes. Red 'x' marks show a clear separation of peaks.</p>





9.3 Optional: Quantitation using Analyst®

LC data analyses can be performed using the Analyst® software as described below. Before starting the process, adjust the retention times (RTs) in the provided quantitation method for Analyst®, see sections 9.3.1 *Adjustment of Retention Times* and 9.2 *Peak Identification*.



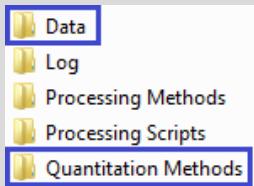
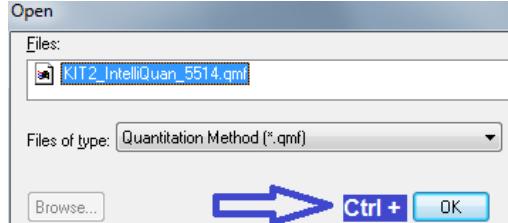
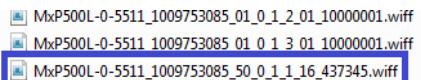
It is recommended to perform the LC data quantitation with MetIDQ™, see previous section 9.1 Quantitation using MetIDQ™.

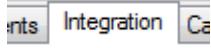
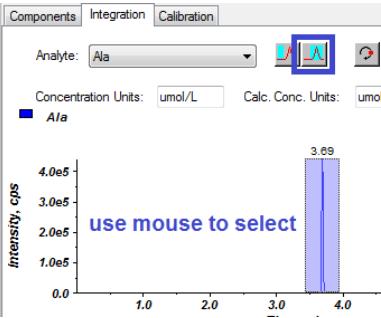
Quantitation Methods for Analyst®

Quantitation methods are provided with the USB stick

<i>MS Instrument</i>	5500 series
<i>LC variant</i>	UHPLC
<i>LC methods</i>	MxP500L-LC1_IntelliQuan_5511.qmf MxP500L-LC2_IntelliQuan_5511.qmf

9.3.1 Adjustment of Retention Times

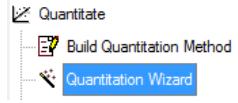
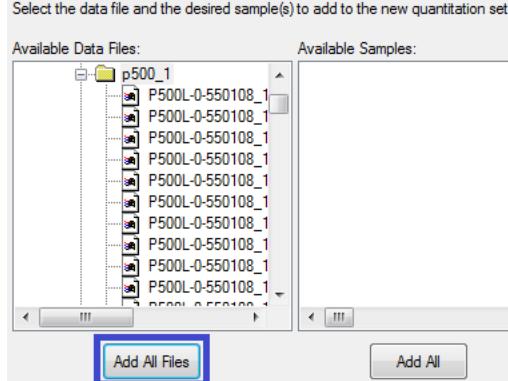
Step	Instructions	Examples
	It is recommended to analyze the data on a different PC than the MS-PC.	
1	Copy the Analyst® project from the MS-PC to the PC for data analysis.	project name: <i>Biocrates MxP500 Kit</i> subproject: <i>2018_03_14_Kit01</i>
	Make sure that all .wiff files are located in the Analyst® project folder “Data” and that the quantitation methods <i>MxP500L-LC1_IntelliQuan_5511.qmf</i> , <i>MxP500L-LC2_IntelliQuan_5511.qmf</i> are in the folder “Quantitation Methods”. Do not rename any .wiff file!	
2	Open the Analyst® quantitation method <i>MxP500L-LC1_IntelliQuan_5511.qmf</i> . 1. File > Open 2. Select the quantitation method, <i>MxP500L-LC1_IntelliQuan_5511.qmf</i> 3. Hold the Ctrl button while clicking OK .	
3	Select a representative MS file. Recommended is QC2, position E2 (well position 50) on the kit plate. The first number after the plate barcode indicates the well position. <u>Example file name of QC2:</u> <i>MxP500L-0-5511_1009753085_50_0</i> _1 _1 _16 _437345.wiff OP-Name_plate barcode_well position_acquisition method_run#_injection#_Sample type ID_sample barcode	

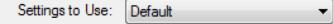
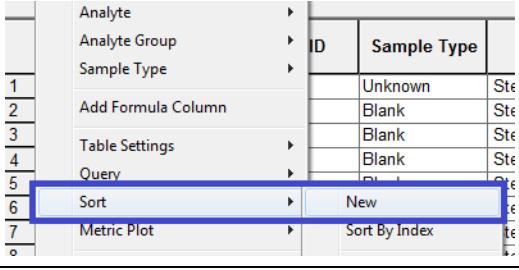
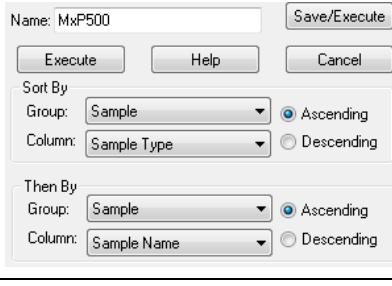
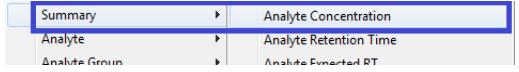
Step	Instructions	Examples								
4	Click on the tab “Integration”.									
5	<p>Review all analytes and check their integration. To update the expected RT</p> <ol style="list-style-type: none"> 1. Select a peak. 2. Click “Select Peak” . 3. If required update the peak integration settings, especially <i>Noise Percent</i>, <i>Peak-Splitting Factor</i>, and <i>Min Peak Height</i>. 	 <table border="1" data-bbox="854 663 1394 827"> <tr> <td>Min Peak Height: 300.000 cps</td> <td>RT Window: 30.000 sec</td> </tr> <tr> <td>Min Peak Width: 0.000 sec</td> <td>Expected RT: 3.686 min</td> </tr> <tr> <td><input type="radio"/> Automatic - IQA II</td> <td>Noise Percent: 90 %</td> </tr> <tr> <td><input checked="" type="radio"/> Specify Parameters - MQ III</td> <td>Peak-Splitting Factor: 1</td> </tr> </table>	Min Peak Height: 300.000 cps	RT Window: 30.000 sec	Min Peak Width: 0.000 sec	Expected RT: 3.686 min	<input type="radio"/> Automatic - IQA II	Noise Percent: 90 %	<input checked="" type="radio"/> Specify Parameters - MQ III	Peak-Splitting Factor: 1
Min Peak Height: 300.000 cps	RT Window: 30.000 sec									
Min Peak Width: 0.000 sec	Expected RT: 3.686 min									
<input type="radio"/> Automatic - IQA II	Noise Percent: 90 %									
<input checked="" type="radio"/> Specify Parameters - MQ III	Peak-Splitting Factor: 1									
!	For peak identification refer to section 9.2 Peak Identification.									
6	Save all changes. Keep the original file name <i>MxP500L-LC1_IntelliQuan_5511.qmf</i> .									
7	C Repeat steps 2 to 6 using the quantitation method <i>MxP500L-LC2_IntelliQuan_5511.qmf</i> .									

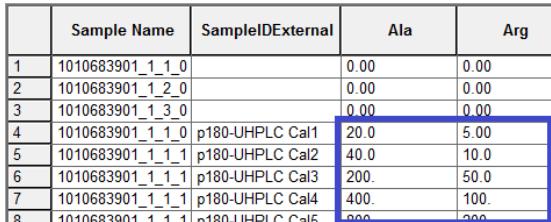
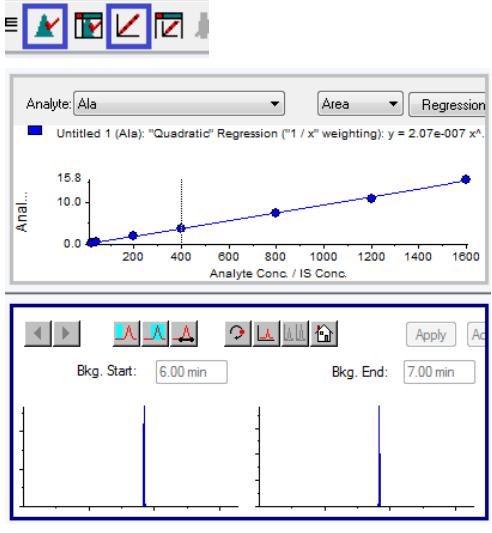
9.4 Quantitation Procedure

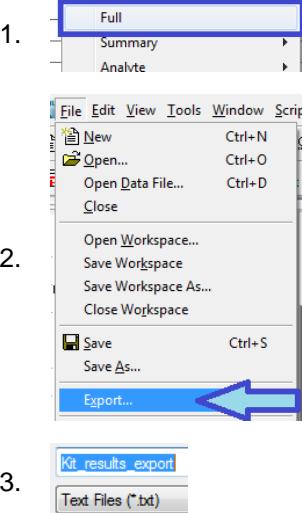


The number format of the PC with Analyst® installed has to be set to “English”, using “.” as decimal separator. If required, set the number format in Windows Control Panel to “English (UK)” or “English (US): **Windows control panel > Region and language > Format**.

Step	Instructions	Examples
	<p>It is recommended to analyze the data on a different PC than the MS-PC. Copy the entire Analyst® project before starting data analysis.</p>	
1	<p>Open the Quantitation Wizard: go to the left panel in the Analyst® software.</p>	
2	<p>Select all .wiff files of the LC1 injections. Click on Add All Files and then on Next.</p>	

Step	Instructions	Examples												
3	Skip the next window with Next .	<p>Please select the settings for the new results table and the default query (if any).</p>  <p>Settings to Use: Default</p> <p>Default Query</p> <p><input checked="" type="radio"/> None</p> <p><input type="radio"/> Select Existing:</p> <p>Query: <input type="text"/> Exec</p>												
4	Select the quantitation method <i>MxP500L-LC1_IntelliQuan_5511.qmf</i> and click Finish .	<p><input checked="" type="radio"/> Choose Existing Method</p> <p>Method: MxP500L-LC1_IntelliQuan_55</p>												
5	<p>When the quantitation process has finished, a table is shown in “Full Layout”.</p> <p>To change the layout for the quantitation process, right-click anywhere in the table and select Sort > New.</p>	 <table border="1"> <thead> <tr> <th>ID</th> <th>Sample Type</th> </tr> </thead> <tbody> <tr><td>Unknown</td><td>St</td></tr> <tr><td>Blank</td><td>St</td></tr> <tr><td>Blank</td><td>St</td></tr> <tr><td>Blank</td><td>St</td></tr> <tr><td>New</td><td>St</td></tr> </tbody> </table>	ID	Sample Type	Unknown	St	Blank	St	Blank	St	Blank	St	New	St
ID	Sample Type													
Unknown	St													
Blank	St													
Blank	St													
Blank	St													
New	St													
6	<p>Use sort criteria as shown on the right.</p> <ol style="list-style-type: none"> Sample: Sample Type and Sample: Sample Name <p>Define a name, e.g. “MxP500”.</p> <p>Click Save/Execute.</p>	 <p>Name: MxP500</p> <p>Save/Execute</p> <p>Execute Help Cancel</p> <p>Sort By</p> <p>Group: Sample Ascending</p> <p>Column: Sample Type Descending</p> <p>Then By</p> <p>Group: Sample Ascending</p> <p>Column: Sample Name Descending</p>												
7	Right click anywhere in the table and select Summary > Analyte Concentration .	 <table border="1"> <thead> <tr> <th>Summary</th> <th>Analyte Concentration</th> </tr> </thead> <tbody> <tr><td>Analyte</td><td>Analyte Retention Time</td></tr> <tr><td>Analyte Group</td><td>Analyte Elapsed RT</td></tr> </tbody> </table>	Summary	Analyte Concentration	Analyte	Analyte Retention Time	Analyte Group	Analyte Elapsed RT						
Summary	Analyte Concentration													
Analyte	Analyte Retention Time													
Analyte Group	Analyte Elapsed RT													

Step	Instructions	Examples																																													
	<p>Copy the concentrations from the Excel file <i>Calibration Standard Concentrations_LC1.xlsx</i> (see USB stick folder “SCIEX\Acquisition and Quantitation Methods\Documents”) into the corresponding fields of calibration standards 1-7.</p>	 <table border="1"> <thead> <tr> <th></th> <th>Sample Name</th> <th>SampleIDExternal</th> <th>Ala</th> <th>Arg</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>1010683901_1_1_0</td> <td></td> <td>0.00</td> <td>0.00</td> </tr> <tr> <td>2</td> <td>1010683901_1_2_0</td> <td></td> <td>0.00</td> <td>0.00</td> </tr> <tr> <td>3</td> <td>1010683901_1_3_0</td> <td></td> <td>0.00</td> <td>0.00</td> </tr> <tr> <td>4</td> <td>1010683901_1_1_0</td> <td>p180-UHPLC Cal1</td> <td>20.0</td> <td>5.00</td> </tr> <tr> <td>5</td> <td>1010683901_1_1_1</td> <td>p180-UHPLC Cal2</td> <td>40.0</td> <td>10.0</td> </tr> <tr> <td>6</td> <td>1010683901_1_1_1</td> <td>p180-UHPLC Cal3</td> <td>200.</td> <td>50.0</td> </tr> <tr> <td>7</td> <td>1010683901_1_1_1</td> <td>p180-UHPLC Cal4</td> <td>400.</td> <td>100.</td> </tr> <tr> <td>8</td> <td>1010683901_1_1_1</td> <td>p180-UHPLC Cal5</td> <td>800.</td> <td>200.</td> </tr> </tbody> </table>		Sample Name	SampleIDExternal	Ala	Arg	1	1010683901_1_1_0		0.00	0.00	2	1010683901_1_2_0		0.00	0.00	3	1010683901_1_3_0		0.00	0.00	4	1010683901_1_1_0	p180-UHPLC Cal1	20.0	5.00	5	1010683901_1_1_1	p180-UHPLC Cal2	40.0	10.0	6	1010683901_1_1_1	p180-UHPLC Cal3	200.	50.0	7	1010683901_1_1_1	p180-UHPLC Cal4	400.	100.	8	1010683901_1_1_1	p180-UHPLC Cal5	800.	200.
	Sample Name	SampleIDExternal	Ala	Arg																																											
1	1010683901_1_1_0		0.00	0.00																																											
2	1010683901_1_2_0		0.00	0.00																																											
3	1010683901_1_3_0		0.00	0.00																																											
4	1010683901_1_1_0	p180-UHPLC Cal1	20.0	5.00																																											
5	1010683901_1_1_1	p180-UHPLC Cal2	40.0	10.0																																											
6	1010683901_1_1_1	p180-UHPLC Cal3	200.	50.0																																											
7	1010683901_1_1_1	p180-UHPLC Cal4	400.	100.																																											
8	1010683901_1_1_1	p180-UHPLC Cal5	800.	200.																																											
8	<p>Right-click anywhere in the table and select Summary > Calculated Concentration.</p>																																														
9	<p>Check the calibration curves and the integration of the peaks. Use the “Calibration – Pane”  and the “Peak Review - Pane” .</p> <p>Manually correct the peak integration settings if necessary. For peak identification refer to section 9.2 Peak Identification.</p> <p>Information: Calibration curves are only available for 7-point calibrated metabolites.</p>																																														

Step	Instructions	Examples
10	<p>For results transfer to Met/DQ™, export the results:</p> <ol style="list-style-type: none"> 1. Right-click anywhere in the table and select Full. 2. Select File > Export... 3. Export the results as “Text Files (*.txt)”. <p>This .txt file will be imported into Met/DQ™ (refer to Met/DQ™ user manual).</p>	
11	 Repeat steps 1 to 10 with LC2 data using the quantitation method <i>MxP500L-LC2_IntelliQuan_5511.qmf</i> .	
		<p>The accuracy of the calculated QC concentrations is checked in Met/DQ™. The accuracies are calculated by Met/DQ™ and visualized in MetVAL after importing the result files (.rdb).</p>



Please refer to the Met/DQ™ user manual section **5.1 LC data – Import Result Files**. To evaluate the results and perform statistics, you can use the Met/DQ™ tool **StatPack**, as well as other tools such as **MetaboAnalyst**.

10 Data Processing – FIA Part

FIA data analysis is performed by the MetIDQ™ software. Go to **MetCONC > FIA** and import the MS data (.wiff) of the FIA1 and FIA2 parts. The procedure is described in the MetIDQ™ user manual in section 5.3 *FIA data – quantitation by MetIDQ™*.



Please refer to the MetIDQ™ Nitrogen user manual section **5.3 FIA data – quantitation by MetIDQ™**. To evaluate the results and perform statistics, you can use the MetIDQ™ tool **StatPack**, as well as other tools such as **MetaboAnalyst**.

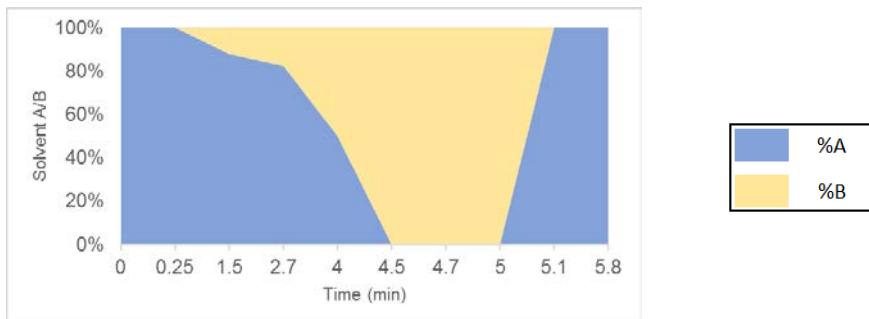
11 Appendix

11.1 LC Settings

11.1.1 UHPLC Gradient

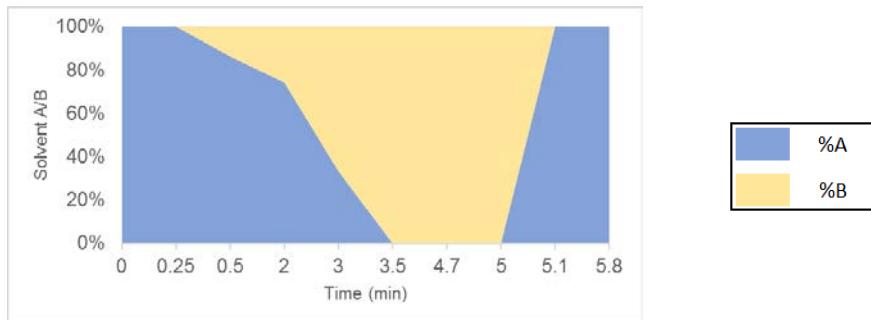
UHPLC Gradient LC1 part for methods *MxP500-LC1_5511.dam*

No	Time (min)	Flow (mL/min)	A (%)	B (%)
1	0.00	0.8	100	0
2	0.25	0.8	100	0
3	1.50	0.8	88	12
4	2.70	0.8	82.5	17.5
5	4.00	0.8	50	50
6	4.50	0.8	0	100
7	4.70	1.0	0	100
8	5.00	1.0	0	100
9	5.10	1.0	100	0
10	5.80	0.8	100	0



UHPLC Gradient LC2 part for methods *MxP500-LC2_5511.dam*

No	Time (min)	Flow (mL/min)	A (%)	B (%)
1	0.00	0.8	100	0
2	0.25	0.8	100	0
3	0.50	0.8	75	25
4	2.00	0.8	50	50
5	3.00	0.8	25	75
6	3.50	0.8	0	100
7	4.70	1.0	0	100
8	5.00	1.0	0	100
9	5.10	1.0	100	0
10	5.80	0.8	100	0

**FIA Gradient** for methods *MxP500F-FIA1_5501.dam* and *MxP500F-FIA2_5501.dam*

No	Time (min)	Flow (mL/min)	A (%)	B (%)
1	0.0	0.03	0	100
2	1.6	0.03	0	100
3	2.4	0.20	0	100
4	2.8	0.20	0	100
5	3.0	0.03	0	100

MxP® Quant 500 Kit for SCIEX triple quadrupole instruments

11.1.2 Column Oven Temperature

Parameter	Value
Temperature	LC: 50 °C FIA: off or 25 °C

11.1.3 Injection Volume

Parameter	Value
Injection volume	LC: 5 µL (addition of 10 µL water) FIA: 20 µL

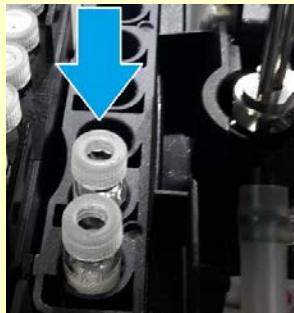
11.1.4 Agilent® LC Settings

Autosampler

Parameter	Value
Wash solvent	25% acetonitrile, 25% methanol, 25% isopropanol, 25% water
Seal wash (if applicable)	10% methanol, 90% water
Syringe size (µL)	40
Injection volume (µL)	LC: 5 FIA: 20
Draw speed (µL/min)	200.0
Eject speed (µL/min)	200.0
Needle Level (mm)	1.0
Temerature control	Enable
Sample temperature (°C)	10
Wash	Enable

Parameter	Value												
Wash location	Flush Port												
Wash time (1-999 sec)	10												
Wash cycles (1-5)	not used												
Wash Vial number	not used												
Wash rack number	not used												
Automatic delay reduction	Disable												
Equilibration time (sec)	2												
Enable vial/well bottom wensing	Enable												
Use custom injection program	LC: Enbale FIA: Disable												
Program table	<p>1: DRAW def. amount from sample, def. speed, def. offset 2: DRAW 1.0 µl from air, def. speed, def. offset 3: WASH NEEDLE in flush port for 10 sec. 4: DRAW 10.0 µl from Vial 1, def. speed, def. offset 5: INJECT</p> <p>Information: Vial 1 used with method <i>MxP500-LC1_5511.dam</i> Vial 2 used with method <i>MxP500-LC2_5511.dam</i></p>												
For line 3 and 4 use these commands:													
 <table border="1" style="margin-left: auto; margin-right: auto;"> <tr> <td style="padding: 5px;">LC1 + LC2</td> <td style="padding: 5px;">Custom Injector Program</td> </tr> <tr> <td style="padding: 5px;"></td> <td style="padding: 5px;"> Line # Function Position Location Time (sec) Cycles Offset (mm) 3 WASH FLUSH PORT Vial 1 10 1 DEF. </td> </tr> <tr> <td style="padding: 5px;">LC1</td> <td style="padding: 5px;">Custom Injector Program</td> </tr> <tr> <td style="padding: 5px;"></td> <td style="padding: 5px;"> Line # Function Amount (µl) Source Location Speed (µl/min) Offset (mm) 4 DRAW 10.0 LOCATION Vial 1 DEF. DEF. </td> </tr> <tr> <td style="padding: 5px;">LC2</td> <td style="padding: 5px;">Custom Injector Program</td> </tr> <tr> <td style="padding: 5px;"></td> <td style="padding: 5px;"> Line # Function Amount (µl) Source Location Speed (µl/min) Offset (mm) 4 DRAW 10.0 LOCATION Vial 2 DEF. DEF. </td> </tr> </table>		LC1 + LC2	Custom Injector Program		Line # Function Position Location Time (sec) Cycles Offset (mm) 3 WASH FLUSH PORT Vial 1 10 1 DEF.	LC1	Custom Injector Program		Line # Function Amount (µl) Source Location Speed (µl/min) Offset (mm) 4 DRAW 10.0 LOCATION Vial 1 DEF. DEF.	LC2	Custom Injector Program		Line # Function Amount (µl) Source Location Speed (µl/min) Offset (mm) 4 DRAW 10.0 LOCATION Vial 2 DEF. DEF.
LC1 + LC2	Custom Injector Program												
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LC1	Custom Injector Program												
	Line # Function Amount (µl) Source Location Speed (µl/min) Offset (mm) 4 DRAW 10.0 LOCATION Vial 1 DEF. DEF.												
LC2	Custom Injector Program												
	Line # Function Amount (µl) Source Location Speed (µl/min) Offset (mm) 4 DRAW 10.0 LOCATION Vial 2 DEF. DEF.												

Parameter	Value
Autosampler settings	<p>LC1 and LC2</p> <p><u>Agilent Autosampler Properties:</u></p> <p>The dialog box shows the following settings:</p> <ul style="list-style-type: none">Acquisition method:<ul style="list-style-type: none">Mass Spec 5.500 minPeriod 5.500 min+MRMIntegrated Valco ValveAgilent 1290 Binary Pump (5.8 mins)Equilibrate (0.0 mins)Run (5.8 mins)Agilent 1290 Infinity AutosamplerAgilent 1290 Thermostatted ColumnAgilent Autosampler Properties tab selected.Advanced Properties tab available.Inject Details:<ul style="list-style-type: none">Syringe Size (μl): 40Injection Volume (μl): 5.0Draw Speed (μl/min): 200.0Eject Speed (μl/min): 200.0Needle Level (mm): 1.0Wash Details:<ul style="list-style-type: none">Enabled: <input checked="" type="checkbox"/>Wash Location: Flush PortWash Time (1 - 999 sec): 10Wash Cycles (1 - 5): 1Wash Vial Number: 1Wash Rack Number: 1Temperature Control:<ul style="list-style-type: none">Enabled: <input checked="" type="checkbox"/>Setpoint (4 - 40 C): 10

Parameter	Value														
Autosampler settings	<p style="text-align: center;">LC1 and LC2</p> <p><u>Advanced Properties – “Sandwich injection” procedure:</u></p> <ul style="list-style-type: none"> • Activate "User Custom Injector Program". • Required commands and parameters are shown below. <div style="border: 1px solid #ccc; padding: 10px;"> <p>Agilent Autosampler Properties Advanced Properties</p> <p>Automatic Delay Volume Reduction <input type="checkbox"/> Enabled Sample Flush-Out Factor: <input type="text" value="5.0"/></p> <p>Sample Flush-Out Factor times Injection Volume equals the volume of mobile phase to pump through the injector before switching the valve to bypass.</p> <p>Equilibration Time (sec): <input type="text" value="2"/></p> <p><input checked="" type="checkbox"/> Enable Vial/Well Bottom Sensing <input checked="" type="checkbox"/> Use Custom Injector Program</p> <p>Custom Injector Program</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th>Line #</th> <th>Function</th> <th>Amount (µl)</th> <th>Source</th> <th>Location</th> <th>Speed (µl/min)</th> <th>Offset (mm)</th> </tr> </thead> <tbody> <tr> <td></td> <td>DRAW</td> <td>DEF.</td> <td>SAMPLE</td> <td>Vial 1</td> <td>DEF.</td> <td>DEF.</td> </tr> </tbody> </table> <p>Program Table</p> <pre> 1: DRAW def. amount from sample, def. speed, def. offset 2: DRAW 1.0 µl from air, def. speed, def. offset 3: WASH NEEDLE in flush port for 10 sec. 4: DRAW 10.0 µl from Vial 1, def. speed, def. offset 5: INJECT </pre> <p style="text-align: right;"> <input type="button" value="Change"/> <input type="button" value="Insert"/> <input type="button" value="Append"/> <input type="button" value="Copy"/> <input type="button" value="Paste"/> <input type="button" value="Delete"/> </p> <div style="background-color: #ffffcc; padding: 10px; margin-top: 10px;"> <p>!</p> <p>Fill two 1.5 mL glass vials with water. Place both vials in the autosampler tray in positions 1 and 2, as shown in the picture.</p> <p>Vial 1 is used with the LC1 method. Vial 2 with the LC2 method.</p>  </div> </div>	Line #	Function	Amount (µl)	Source	Location	Speed (µl/min)	Offset (mm)		DRAW	DEF.	SAMPLE	Vial 1	DEF.	DEF.
Line #	Function	Amount (µl)	Source	Location	Speed (µl/min)	Offset (mm)									
	DRAW	DEF.	SAMPLE	Vial 1	DEF.	DEF.									

Parameter	Value
Autosampler settings	<p style="text-align: center;">FIA1 and FIA2</p> <p>The screenshot shows the 'FIA1 and FIA2' configuration window. On the left, there's a tree view of the acquisition method, starting with 'Acquisition Method' which includes 'Mass Spec 5.500 min' and 'Period 5.500 min'. Below that is 'Integrated Valco Valve' with 'Agilent 1290 Binary Pump (5.8 mins)' and its sub-options 'Equilibrate (0.0 mins)' and 'Run (5.8 mins)'. At the bottom is 'Agilent 1290 Infinity Autosampler' and 'Agilent 1290 Thermostatted Column'. To the right of the tree are two main panels: 'Agilent Autosampler Properties' and 'Advanced Properties' (which is currently not selected). Under 'Agilent Autosampler Properties', there are sections for 'Inject Details' (Syringe Size: 40, Injection Volume: 20.0), 'Wash Details' (Enabled checked, Wash Location: 'Flush Port'), and 'Temperature Control' (Enabled checked, Setpoint: 10). Other fields like 'Draw Speed', 'Eject Speed', 'Needle Level', 'Wash Time', 'Wash Cycles', 'Wash Vial Number', and 'Wash Rack Number' also have their respective values displayed.</p>

Column Compartment

Parameter	Value
Left temperature (°C)	50.0
Right temperature (°C)	50.0
Temperature tolerance +/- (°C)	1.0
Start acquisition tolerance +/- (°C)	0.5

Binary Pump

Parameter	Value
Mixer	Jet Weaver V35 Mixer
Equilibration (min)	0.0
Minimum pressure (psi)	0.0
Maximum pressure (psi)	17404.0
Stroke volume (μL)	-1.0
Maximum flow ramp up (mL/min ²)	100.0
Maximum flow ramp down (mL/min ²)	100.0
Left stroke volume (μl)	-1.0
Right stroke volume (μl)	0.0
Left solvent (recommendation)	LC: A1 FIA: A2
Right solvent (recommendation)	LC: B1 FIA: B2

11.2 MS Settings

	Acquisition method
LC1	MxP500L-LC1_5511.dam
LC2	MxP500L-LC2_5511.dam
FIA1	MxP500F-FIA1_5501.dam
FIA2	MxP500F-FIA2_5501.dam

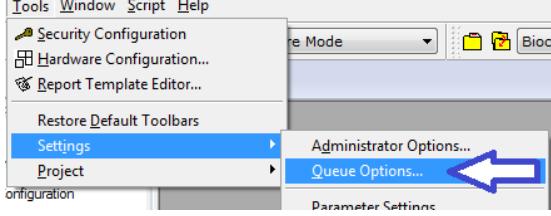
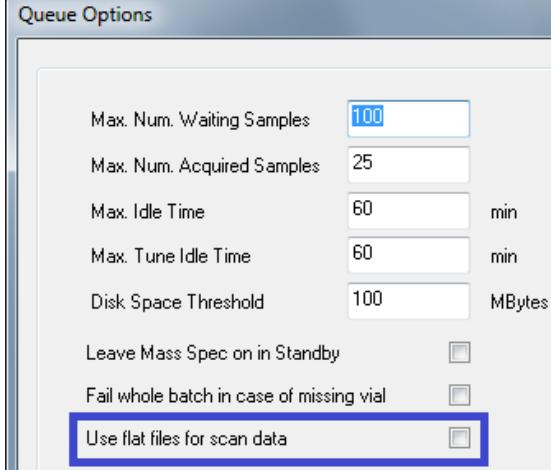
Acquisition method →		LC1	LC2	FIA1	FIA2
Option	Parameter	Value	Value	Value	Value
MS	Experiment	1	1	1	1
	Scan type	MRM (MRM)	MRM (MRM)	MRM (MRM)	MRM (MRM)
	Polarity	Positive	Negative	Positive	Positive
	Scheduled MRM	Enable, Basic	Enable, Basic	Disable	Disable
	MRM detection window (sec)	30	30	--	--
	Target scan time (sec)	0.15	0.15	--	--
	Duration (min)	5.45	5.45	2.95	2.95
	Delay Time (sec)	0	0	0	0
	Cycle (sec)	0.15	0.15	N/A in unscheduled mode	
Advanced MS	Resolution Q1	Unit	Unit	Unit	Unit
	Resolution Q3	Unit	Unit	Unit	Unit
	Intensity threshold (total count)	0	0	0	0
	Settling time (ms)	0	0	0	0
	Pause between mass ranges (ms)	2	2	5.007	3
Source/ Gas	Curtain gas (CUR)	45.0	20.0	20.0	10.0
	Collision gas (CAD)	9	8	9	9
	Ion spray voltage (IS)	5500.0	-4500.0	5500.0	5500.0
	Temperature (TEM)	500.0	650.0	200.0	350.0
	Ion source gas 1 (GS1)	60.0	40.0	40.0	30.0
	Ion source gas 2 (GS2)	70.0	40.0	50.0	90.0



Double-check the parameter **Pause between mass ranges (ms)**.

11.3 Analyst® Settings

For MS data acquisition two options are available using the Analyst® software. To create one file (.wiff) per injection, deactivate the option “Use flat files for scan data”. To activate this option follow the steps below.

Step	Instructions	Examples
1	Start Analyst® and activate the <i>Configure</i> mode.	
2	Select Tools > Settings > Queue Options...	
3	<p>Deactivate the option “Use flat files for scan data”.</p> <p>Click “OK”.</p>	

11.4 Abbreviations

µL	Microliter
bar	Bar
Cal	Calibration standard
ESI	Electrospray ionization
FIA	Flow injection analysis
h	Hour
ISTD	Internal standard
LC-MS	Instrument combination of UHPLC pump and SCIEX mass spectrometer
min	Minutes
mL	Milliliter
MxP®	Metabolomics profiling
MS	Mass spectrometer
MS-PC	PC that controls the mass spectrometer instrument
MRM	Multiple reaction monitoring
QC	Quality control
RT	Retention time
sec	Seconds
UHPLC	Ultra high performance liquid chromatography

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