

Nov 14, 2025

F5 Targeted profiling method for metabolomics

This protocol is a draft, published without a DOI.

Megan Danielewicz¹

¹Stanford



Megan Danielewicz

Stanford

OPEN  ACCESS



Protocol Citation: Megan Danielewicz 2025. F5 Targeted profiling method for metabolomics. **protocols.io**

<https://protocols.io/view/f5-targeted-profiling-method-for-metabolomics-c46byzan>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: November 10, 2023

Last Modified: November 14, 2025

Protocol Integer ID: 91043

Keywords: profiling method for metabolomic, metabolomic, targeted profiling method, f5, preparation of reagent, extraction of plasma, reagent

Abstract

This protocol details the preparation of reagents and samples and extraction of plasma.

Attachments



[n295b9mpf.docx](#)

1.8MB

Materials

Analytes, reagents and assay materials:

Details for ordering the appropriate materials for metabolomic analysis are provided in this section. To order materials; the supplier's name, contact information, and the part number for each reagent or piece of equipment required are indicated below:

Chemicals and reagents:

	A	B	C
	Supplier	Description	Part Number
	Sigma	Water-HPLC grade	34877-4L
	Sigma	Methanol-HPLC grade	646377-4L
	Sigma	Acetonitrile-HPLC grade	34998-4L
	Thermo Fisher	Formic Acid-LCMS grade	85178

Equivalent reagents from other suppliers can also be used. Assay result might deviate from this optimized method if using reagents other than the ones suggested in this SOP.

Metabolite standards:

	A	B	C
	Supplier	Description	Part Number
	Cambridge Isotopes	METABOLOMICS QRESS STANDARD 1	MSK-QRESS1-1
		METABOLOMICS QRESS STANDARD 2	MSK-QRESS2-1

The above listed standards are labeled internal standards recommended to order to cover wide ranges of metabolites during method development. They are not mandatory for the assay, but we recommend a labeled internal standard to track extractions.

HPLC columns:

	A	B	C
	Supplier	*Description	Part number



	A	B	C
	Phenomenex http://www.phenomenex.com/	Kinetex, 2.6μM F5 100 A (150× 2.1 mm)	00F-4723-ANY0

Troubleshooting



Preparation of reagents and solutions

1 Mobile Phase and Sample prep

The instructions for preparing each reagent/solution are provided below:

1.1 **Mobile phase A (0.1% formic acid in water):**



Mix  1 mL of formic acid with  999 mL of water in a 1 L bottle.





1.2 **Mobile phase B (0.1% formic acid in acetonitrile):**



Mix  1 mL of formic acid with  999 mL of methanol in a 1 L bottle.

1.3 **Needle rinse (1:1:1:1 water/methanol/iso-propanol/acetonitrile):**







Mix  250 mL of water,  250 mL of methanol,  250 mL of isopropanol and  250 mL of acetonitrile in a 1L bottle.

Sample and Control Preparation

2 The instructions for preparing the double blank, blank and QC samples are listed below:

2.1 **CIL QRESS Internal standard stock solution prep**



- QReSS vial 1 Stock: Add  1 mL of 50% methanol via a needle to the stoppered vial to reconstitute (do not remove stopper prior to reconstitute).
- QReSS vial 2 Stock: Add  1 mL of 50% methanol via a needle to the stoppered vial to reconstitute (do not remove stopper prior to reconstitute).
- Take  10.25 μ L of each QReSS vial 1 and vial 2 and add  979.5 μ L of methanol.

2.2 **Double blank sample:**








Pipet  1 mL 1:1 water/methanol into an autosampler vial.

2.3 **Blank sample:**



Add  50 μL water to  390 μL methanol and  10 μL of internal standard stock solution.

2.4 **Pooled sample for method development:**

Sample extracts containing internal standards can be used.

Extraction protocol

10s

3 Add sample into 1.5 mL eppendorf tube:

3.1 Mouse organ - 100 mg minimum (or closest you can come to that)

3.2 Plasma – 100 μL minimum - Make sure the plasma is settled in the bottom of the tube but not on the walls of the tube.

3.3 Bacterial supernatant/spent/fresh media – 100 μL -- may need to dilute later

3.4 Brine or Food - 100 μL or 100 mg

3.5 ALWAYS MAKE – A Double blank – An HPLC vial with 1 mL Mobile phase A (no sample processing)


3.6 ALWAYS MAKE – An extraction blank -- process an empty tube alongside your samples



3.7 ALWAYS MAKE – An ISTD blank – a blank with only an ISTD spike processed alongside your samples Add 20 μL of "QReSS ISTD spiking stock". Always want to add it before extraction to normalize for what happens to the sample during extraction


4 Add  1000 μL of methanol (preferably ice cold)

4.1 Add +  20 μL of QReSS IS spiking stock solution



- 5 If your sample is a solid, perform bead beating for 1 min, chilling every 30 seconds. After bead beating chill for 30 min.
- 6 Vortex the sample/ solvent mixture for  00:00:10 . 10s
- 7 Chill for 10+ min in freezer. Can chill overnight.
- 8 Centrifuge the sample/ solvent mixture at  10000 rcf, 8°C, 00:10:00 . 10m

- 9 Transfer supernatant to fresh labeled eppendorf.
- 9.1 If you are using an aliquot of this for the NPH derivatization, take it now!!!
- 9.2 NPH aliquot: Add 50 uL to a second labeled Eppendorf.
- 10 Optional step: Chill  4 °C for  02:00:00 for any further precipitation of proteins. 2h
- 11 Dry down supernatant via speedvac or nitrogen evaporation (preferred)
- 12 Reconstitute in  100 µL 50% MeOH .
- 13 Centrifuge the sample/solvent mixture again at  15000 rcf, 00:10:00 (can use a centrifuge filter). 10m

- 14 Transfer 50 uL of solution to fresh labeled HPLC vial with insert.
- 15 Inject  5 µL of the supernatant for LC-MS analysis.

Gradient conditions

16

Pump
Autosampler
Oven
System Controller

B.GE1

Stop time: 20.00 min

☒ B.Conc
☐ A.Conc

Flow: 0.2000 mL/min
Time to reach the flow: 1.00 min
A.Conc: 100.0 %
B.Conc: 0.0 % B.Curve: 0

Pressure limits
Minimum: 0 psi Maximum: 13000 psi

Gradient
☒ Advanced
☐ Simple

	Time	Flow	A.Conc	B.Conc	B.Curve
1		0.2000	100.0	0.0	0
2	2.10	0.2000	100.0	0.0	0
3	14.00	0.2000	5.0	95.0	0
4	16.00	0.2000	5.0	95.0	0
5	16.10	0.2000	100.0	0.0	0
6	20.00	0.2000	100.0	0.0	0
7					

- Mobile phase settings

- Gradient start adjustment

Injection Volume 5.0 µL

Pump
Autosampler
Oven
System Controller

Column Oven
A: AE Column Oven

☒ Column Oven A

Oven temperature: 30 °C
Temperature limit(Maximum): 105 °C
Ready check: Off

- Oven A - Advanced

MX_F5_20min (Metabolite_Library) x

IntegratedSystem : ExionLC AE

Valve : Valve Model

Time (min)	Position
0	B
0.5	A

Source Conditions

17

QRE5 F5 method - Copy (Metabolite_Library) x

Method Overview

Device: SCIEX 7500+ system
Ion Source: E-ANLYT 200+ uL

Add Experiment

sMRM

0 min - 20 min

sMRM

0 min - 20 min

Method duration: 20 min
Minimum dwell time: 3 ms
Target cycle time: 1000 ms
Maximum dwell time: 250 ms

Source and Gas Parameters

Ion source gas 1: 30 psi
Ion source gas 2: 50 psi
Curtain gas: 40 psi
CAD gas: 9 psi
Source temperature: 350 °C

Experiment: MRM

Polarity: Negative
Spray voltage: 1600 V

Advanced Experiment Settings

Setting time: 15 ms
Pause time: 4 ms

Mass Table: Import
MRM Mode: Scheduled MRM
Apply sMRM triggering: ☐
Q0 dissociation: Simple

Group ID	Compound ID	Retention time (min)	Retention time tolerance (+/-, s)	Q1 mass (Da)	Q3 mass (Da)	Edit dwell time	Dwell time (ms)	EP (V)	CE (V)	CKP (V)	Q1 resolution	Q3 resolution
1	QRE5 IS 13C3-Alanine N1	1.63	60	92.000	92.000	<input type="checkbox"/>	40.695	-10.0	-10.0	-11.0	Unit	Unit
2	QRE5 IS D3-Creatinine N1	1.75	60	115.100	41.000	<input type="checkbox"/>	40.640	-10.0	-30.0	-11.0	Unit	Unit
3	QRE5 IS D3-Creatinine N2	1.75	60	115.100	68.000	<input type="checkbox"/>	40.640	-10.0	-20.0	-11.0	Unit	Unit
4	QRE5 IS 15NS-Guanosine N1	7.47	60	287.100	155.000	<input type="checkbox"/>	37.884	-10.0	-20.0	-11.0	Unit	Unit
5	QRE5 IS 15NS-Guanosine N2	7.47	60	287.100	137.000	<input type="checkbox"/>	37.822	-10.0	-35.0	-11.0	Unit	Unit
6	QRE5 IS 15NS-Guanosine N3	7.47	60	287.100	111.100	<input type="checkbox"/>	37.771	-10.0	-40.0	-11.0	Unit	Unit
7	QRE5 IS 13C5-Hypoxanthine N1	3.30	60	140.100	96.000	<input type="checkbox"/>	31.300	-10.0	-23.0	-11.0	Unit	Unit
8	QRE5 IS 13C5-Hypoxanthine N2	3.32	60	140.100	68.000	<input type="checkbox"/>	31.462	-10.0	-35.0	-11.0	Unit	Unit
9	QRE5 IS 13C6-Phenylalanine N1	7.56	60	170.100	109.100	<input type="checkbox"/>	35.527	-10.0	-20.0	-11.0	Unit	Unit
10	QRE5 IS 13C6-Phenylalanine N2	7.56	60	170.100	72.000	<input type="checkbox"/>	35.527	-10.0	-20.0	-11.0	Unit	Unit

The screenshot displays the QRESS software interface for configuring a mass spectrometry method. The main window is titled "QRESS f5 method - Copy (Metabolite Library)".

Method Overview:

- Method duration: 20 min
- Target cycle time: 1000 ms
- Minimum dwell time: 3 ms
- Maximum dwell time: 250 ms

Source and Gas Parameters:

- Ion source gas 1: 30 psi
- Ion source gas 2: 50 psi
- Curtain gas: 40 psi
- CAD gas: 9 psi
- Source temperature: 350 °C

Experiment:

- Experiment type: MRM
- Polarity: Positive
- Spray voltage: 1800 V
- Advanced Experiment Settings:
 - Settling time: 15 ms
 - Pause time: 4 ms
- Mass Table: Impact (MRM Mode: Scheduled MRM)

Mass Table:

Group ID	Compound ID	Retention time (min)	Retention time tolerance (±) (s)	Q1 mass (Da)	Q3 mass (Da)	Edit dwell time	Dwell time (ms)	EP (V)	CE (V)	EXP (V)	Q1 resolution	Q3 resolution
1	QRESS IS 13C3-15N Alanine 1	1.42	60	94.090	47.000	<input type="checkbox"/>	10.000	10.0	17.0	10.0	Unit	Unit
2	QRESS IS 13C3-15N Alanine 2	1.63	60	94.090	29.100	<input type="checkbox"/>	10.000	10.0	50.0	10.0	Unit	Unit
3	QRESS IS 13C4 Putrescine 1	2.00	60	93.100	76.200	<input type="checkbox"/>	10.000	10.0	15.0	10.0	Unit	Unit
4	QRESS IS 13C4 Putrescine 2	2.00	60	93.100	31.000	<input type="checkbox"/>	10.000	10.0	30.0	10.0	Unit	Unit
5	QRESS IS 13C4 Putrescine 3	2.00	60	93.000	59.000	<input type="checkbox"/>	10.000	10.0	25.0	10.0	Unit	Unit
6	QRESS IS D3-Creatinine 1	1.73	60	117.100	47.000	<input type="checkbox"/>	10.000	10.0	30.0	10.0	Unit	Unit
7	QRESS IS D3-Creatinine 4	1.73	60	117.100	89.000	<input type="checkbox"/>	10.000	10.0	20.0	10.0	Unit	Unit
8	QRESS IS D4-Ethanolamine 4	1.55	60	65.900	65.900	<input type="checkbox"/>	10.000	10.0	5.0	10.0	Unit	Unit
9	QRESS IS D4-Ethanolamine 3	1.53	60	65.900	46.100	<input type="checkbox"/>	10.000	10.0	15.0	10.0	Unit	Unit

Right Panel:

- Queue:** Acquisition sample editing, Acquisition sample time remaining, Acquisition queue time remaining.
- Devices:**
 - Extant AE
 - Pump A
 - Pump B
 - Autosampler
 - Oven A
 - System Controller
 - SCIEX 7500 L system LM
 - Syringe Pump Model
 - Valve Model
- Direct Control:**
 - Stability
 - Equilibrate

MRM list can be provided upon request.
QRESS MRM and RT can be provided upon request.

Protocol references

<https://www.biorxiv.org/content/10.1101/2023.11.02.565382v1.full>