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F5 Targeted profiling method for metabolomics

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Protocol status: Working
We use this protocol and it's working

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Abstract

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

Attachments

DOCX

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 µL of each QReSS vial 1 and vial 2 and add 979.5 µL 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 uL 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 uL -- may need to dilute later

3.4 Brine or Food - 100 uL 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 uL 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 beat 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

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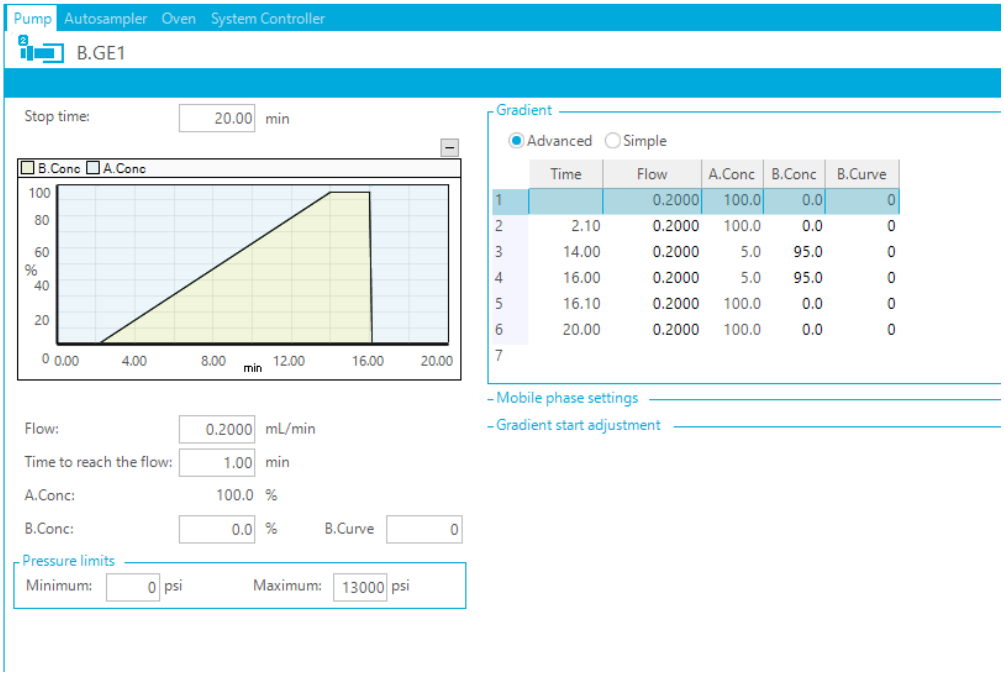

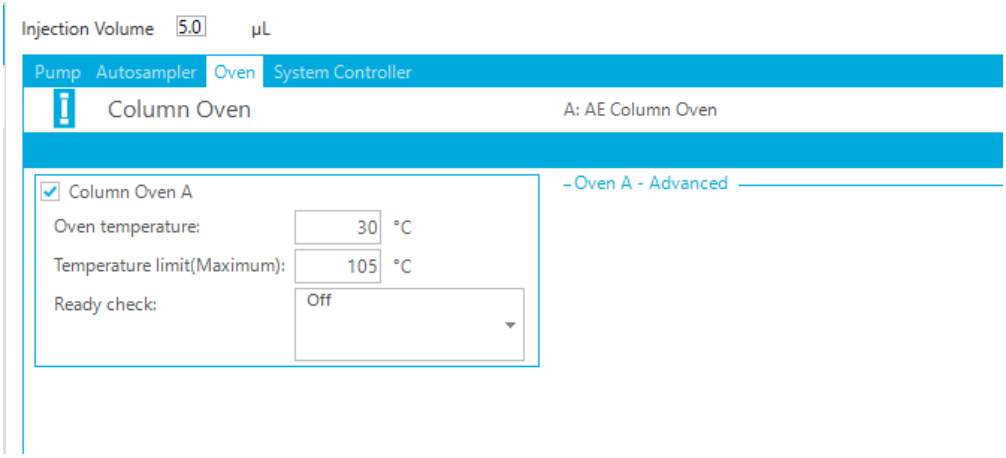

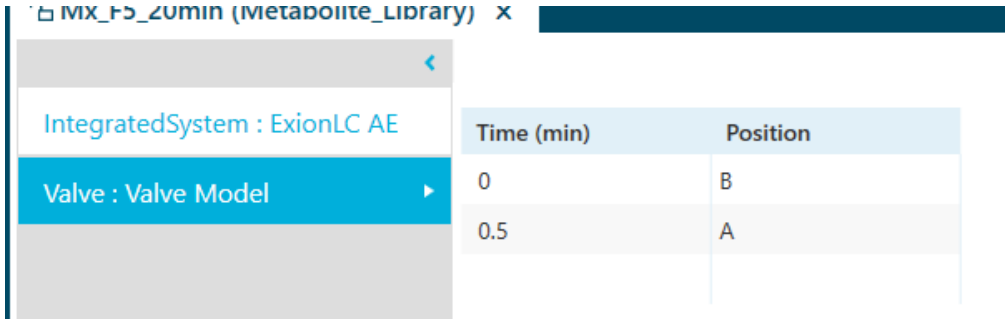

Source Conditions

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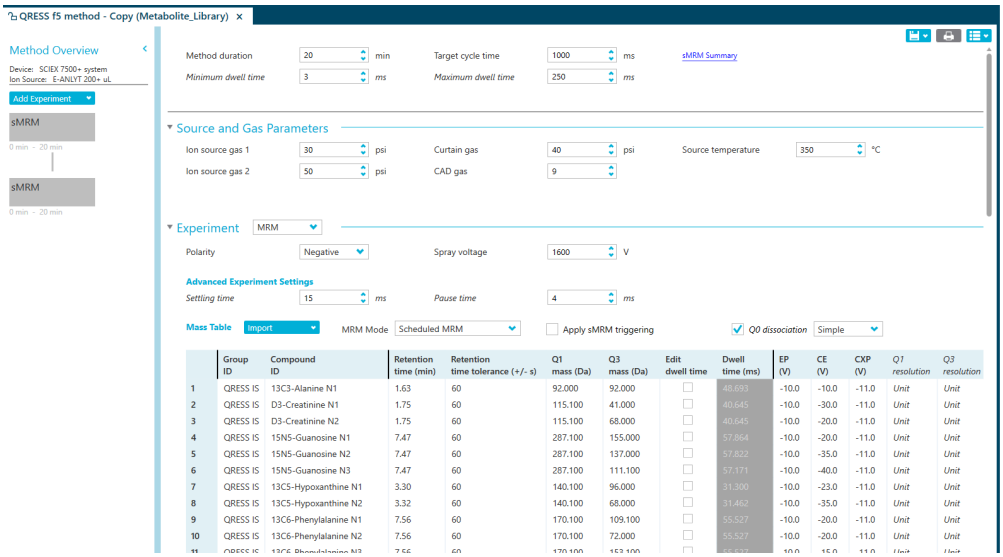
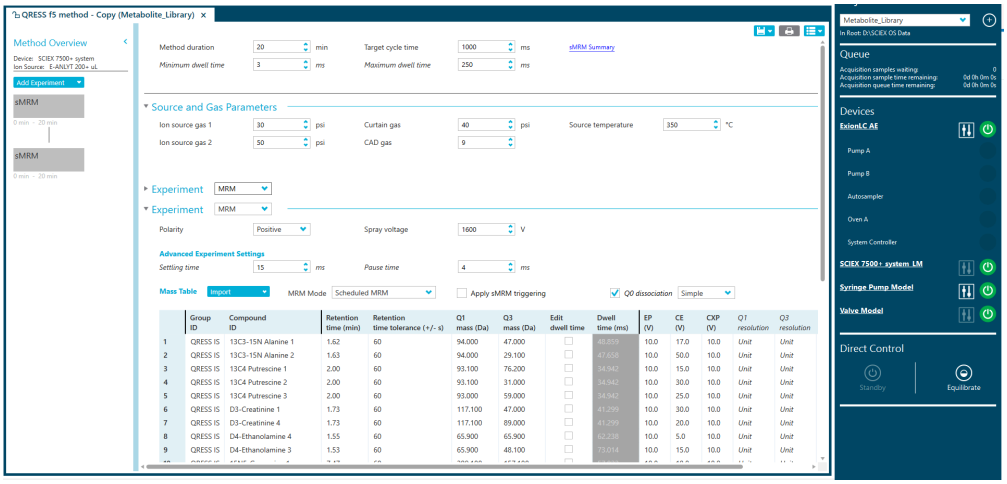


	Group ID	Compound ID	Retention time (min)	Retention time tolerance (+/- s)	Q1 mass (Da)	Q3 mass (Da)	Edit dwell time	Dwell time (ms)	EP (V)	CE (V)	CXP (V)	Q1 resolution	Q3 resolution
1	QRESS IS	13C3-Alanine N1	1.63	60	92.000	92.000		48.693	-10.0	-10.0	-11.0	Unit	Unit
2	QRESS IS	D3-Creatinine N1	1.75	60	115.100	41.000		40.645	-10.0	-30.0	-11.0	Unit	Unit
3	QRESS IS	D3-Creatinine N2	1.75	60	115.100	68.000		40.645	-10.0	-20.0	-11.0	Unit	Unit
4	QRESS IS	15N5-Guanosine N1	7.47	60	287.100	155.000		57.864	-10.0	-20.0	-11.0	Unit	Unit
5	QRESS IS	15N5-Guanosine N2	7.47	60	287.100	137.000		57.822	-10.0	-35.0	-11.0	Unit	Unit
6	QRESS IS	15N5-Guanosine N3	7.47	60	287.100	111.100		57.171	-10.0	-40.0	-11.0	Unit	Unit
7	QRESS IS	13C5-Hypoxanthine N1	3.30	60	140.100	96.000		31.300	-10.0	-23.0	-11.0	Unit	Unit
8	QRESS IS	13C5-Hypoxanthine N2	3.32	60	140.100	68.000		31.462	-10.0	-35.0	-11.0	Unit	Unit
9	QRESS IS	13C6-Phenylalanine N1	7.56	60	170.100	109.100		55.527	-10.0	-20.0	-11.0	Unit	Unit
10	QRESS IS	13C6-Phenylalanine N2	7.56	60	170.100	72.000		55.527	-10.0	-20.0	-11.0	Unit	Unit



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