

Poster Reprint

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A Three-in-One End-to-End Automated Sample Preparation and LC/MS Metabolomics, Lipidomics, and Proteomics Workflow for Plasma

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Introduction

LC/MS omics analyses, comprising metabolomics, lipidomics, and proteomics, offer a way to measure biological changes. This includes measuring components of the three main metabolic branches: (1) conversion of nutrients into energy, predominantly ATP, (2) conversion of nutrients into building blocks, including those used to build proteins and nucleic acids, and (3) conversion of nutrients into waste products.

Comprehensive LC/MS metabolomics, lipidomics and proteomics analysis requires extraction, separation, and detection of all three compound classes from individual samples. We developed a novel automated workflow for extraction of metabolites, lipids and proteins from 20 μL plasma samples and analyzed each extracted fraction with a unique LC/MS method. The coverage and reproducibility across metabolites, lipids, and proteins for this three-in-one omics workflow were assessed.

Experimental

Sample Preparation

Metabolite, lipid and protein fractions were extracted from 20 μL pooled mouse plasma samples using a Bravo Liquid Handler with commercial accessories and VWorks automation protocols.

Proteins were collected and prepared via tryptic digestion using an AssayMap Bravo. Metabolites and lipids were separated by passing the samples through a solid phase extraction plate, metabolites being collected in the flow-through and lipids being captured by the sorbent and subsequently eluted.¹⁻⁴

LC/MS and Software Analysis

Each separate sample fraction was analyzed using targeted or untargeted LC/MS analysis.⁵ Note: it is possible to analyze all three fractions on a single LC/TQ system, switching only the LC/MS methods and washing the LC in between each analysis.⁵⁻⁷

For the current workflow, polar metabolite analysis used HILIC chromatography and a 6495C LC/TQ.⁵ Lipid analysis used RP C18 chromatography and a 6495C LC/TQ.⁶ Protein analysis used RP chromatography and a 6546 LC/Q-TOF. Data analysis utilized commercially available quantitative software and Spectrum Mill software.

Each sample fraction was analyzed for sample prep and LC/MS reproducibility. The lipid fraction was preliminarily analyzed for lipid recovery using ^2H -labeled lipids.

Experimental

The Three-in-One End-to-End Metabolomics, Lipidomics, and Proteomics Workflow

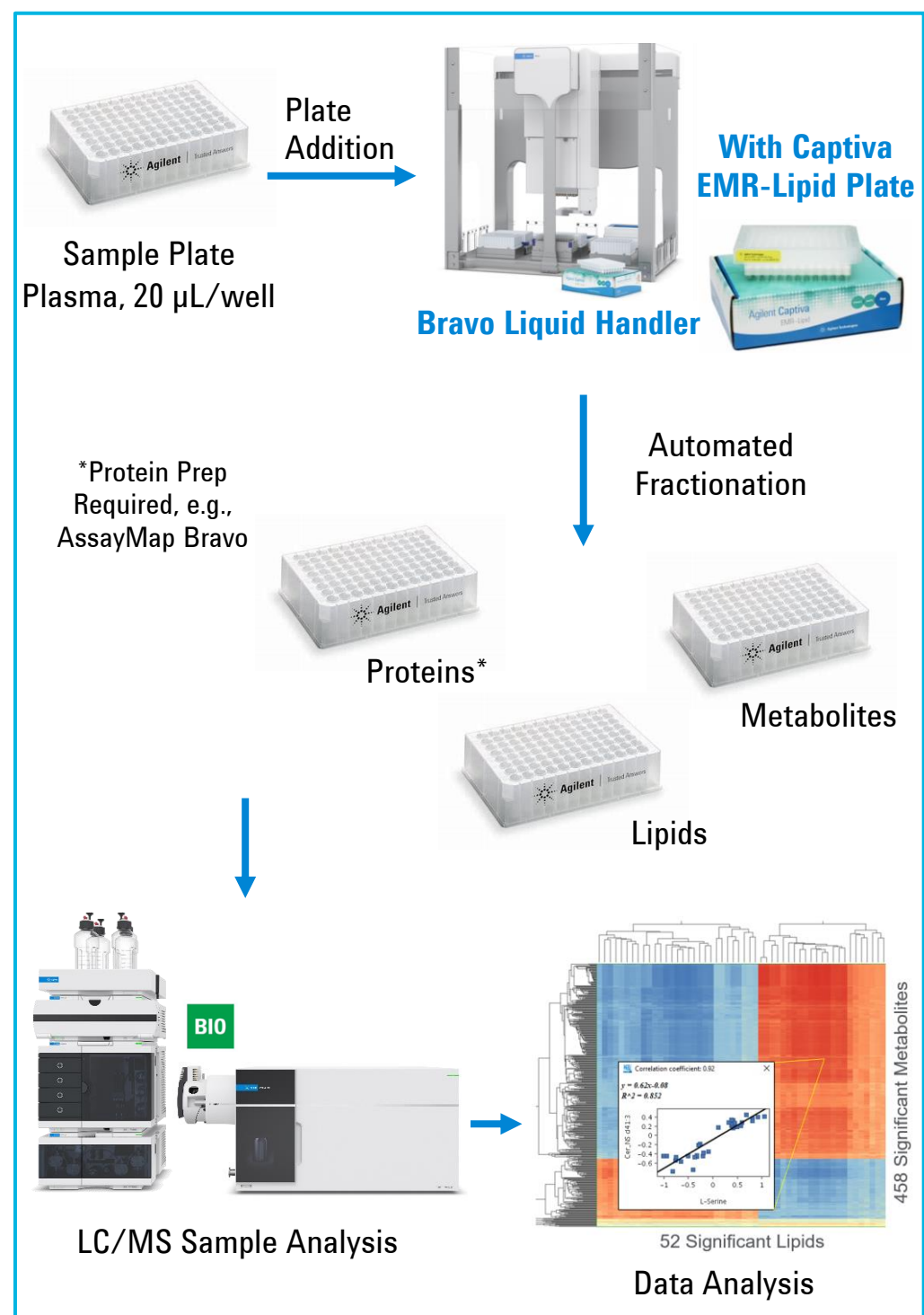


Figure 1. Workflow, from sample preparation to LC/MS analysis. Automated sample prep offers easy-to-implement and reproducible fractionation of metabolites, lipids and proteins. Targeted metabolomic, lipidomic and proteomic analyses can be completed on a single LC/TQ system.

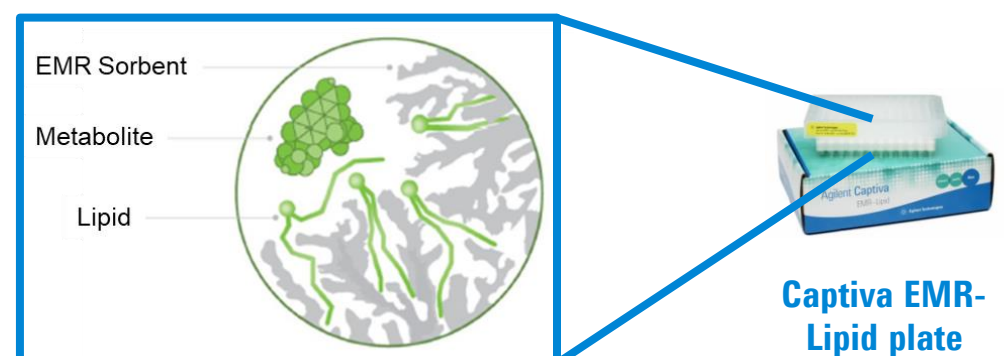


Figure 2. The Captiva EMR-Lipid plate captures lipids and elutes metabolites. After metabolite elution and a plate wash, lipids are eluted by switching to a lipid-elution solvent.¹

Three-in-One Workflow Extracts Metabolites with High Reproducibility and Broad Coverage

Targeted metabolomics analysis detected 437 metabolites, covering major metabolite classes and metabolic pathways.

Table 1. In a subset of 47 metabolites, 70% exhibited total %RSDs $\leq 10\%$, and 87% exhibited total %RSDs $< 20\%$, indicating excellent reproducibility across the end-to-end workflow. Bravo sample prep %RSD equals: %RSD, Total minus %RSD LC/MS (pooled QC).

Analyte	%RSD, Total	%RSD, LC/MS	%RSD, Bravo
2-Deoxyuridine	5	5	0
3-Carboxypropyl Trimethylammonium	10	9	1
4-Guanidobutyric Acid	10	10	1
4-Hydroxyphenylpyruvic Acid	12	9	3
AMP	17	14	4
5-Methoxytryptamine	23	15	9
ADP	29	20	10
Adipic Acid	6	6	0
ADMA	6	7	0
Allantoin	10	8	2
alpha-Ketoglutaric Acid	5	5	0
B-Alanine	9	10	0
B-Ureidoisobutyric Acid	6	6	1
Butyric Acid	8	7	1
Cyclic-AMP	7	7	0
Cholic Acid	8	8	0
cis-Aconitic Acid	19	19	1
Creatine	34	30	4
Creatine Phosphate	16	13	4
Creatinine	9	9	1
Cytidine	7	7	0
2-Deoxycytidine	8	8	0
DL-2-Aminoadipic Acid	16	12	5
D-Pantothenic acid	8	9	0
Glutathione (Oxidized)	44	33	12
Hippuric Acid	5	4	1
Hydroquinone	7	6	2
Hydroxyphenyllactic Acid	9	8	1
Hypotaurine	28	20	8
Indoxyl Sulfate	7	7	1
Kynurenic Acid	7	7	0
L-Glutamic Acid	5	5	1
L-Glutamine	3	4	0
L-Isoleucine	8	8	0
L-Leucine	7	7	0
L-Phenylalanine	8	8	0
L-Tryptophan	13	11	3
L-Tyrosine	17	14	4
N-Acetyl-L-Asparagine	8	10	0
N-Acetyl-DL-Tryptophan	8	9	0
Phosphocholine	11	9	2
Pyroglutamic Acid	9	7	3
Riboflavin	7	7	1
Thiamine	6	6	1
trans-4-Hydroxy-L-Proline	4	4	0
Uric Acid	54	33	22
Uridine	6	7	0

Three-in-One Workflow Extracts Proteins with High Reproducibility and Broad Coverage

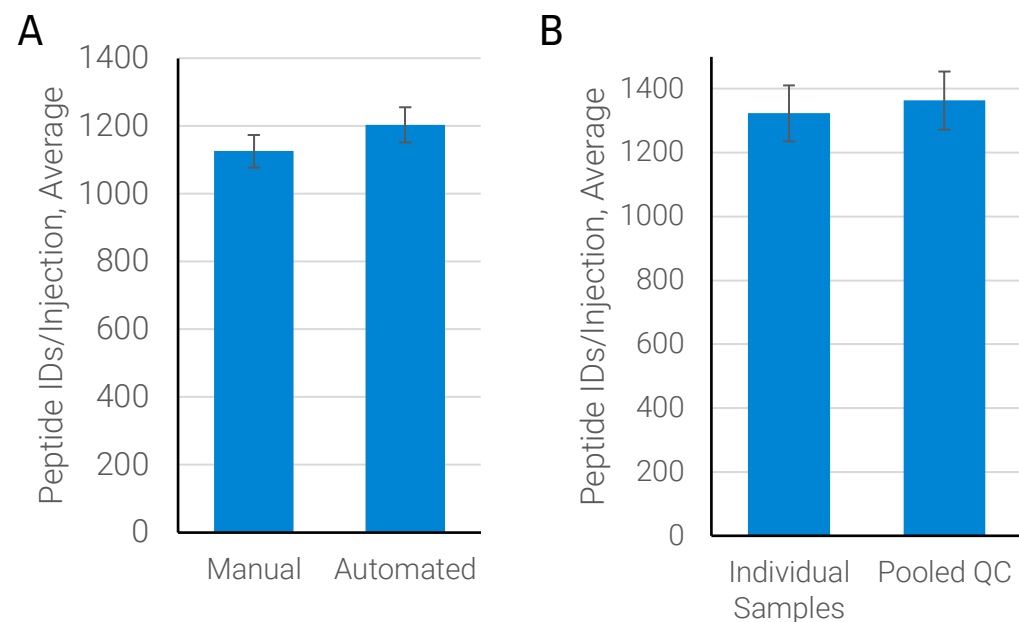


Figure 3. (A) A larger number of peptides were identified for automated (1204, $n = 8$) versus manual (1126, $n = 8$) protein pellet extraction from 20 μL plasma samples, Student's t -test, $p = 0.0077$. (B) An equivalent number of peptides were identified for individually prepared samples (1323, $n = 24$) and a pooled QC sample injected repeatedly (1363, $n = 11$). Student's t -test, $p = 0.26$. Error bars are SD.

Table 2. Reproducibility of automated peptide extraction from 20 μL plasma samples. %RSD for peptide identifications was calculated across 24 samples (%RSD, Total) and multiple injections of a pooled QC (%RSD, LC/MS), the difference between the two indicating the %RSD for sample prep using the Bravo Liquid Handler.

Analyte	%RSD, Total	%RSD, LC/MS	%RSD, Bravo
Peptide #	6.63	6.70	0

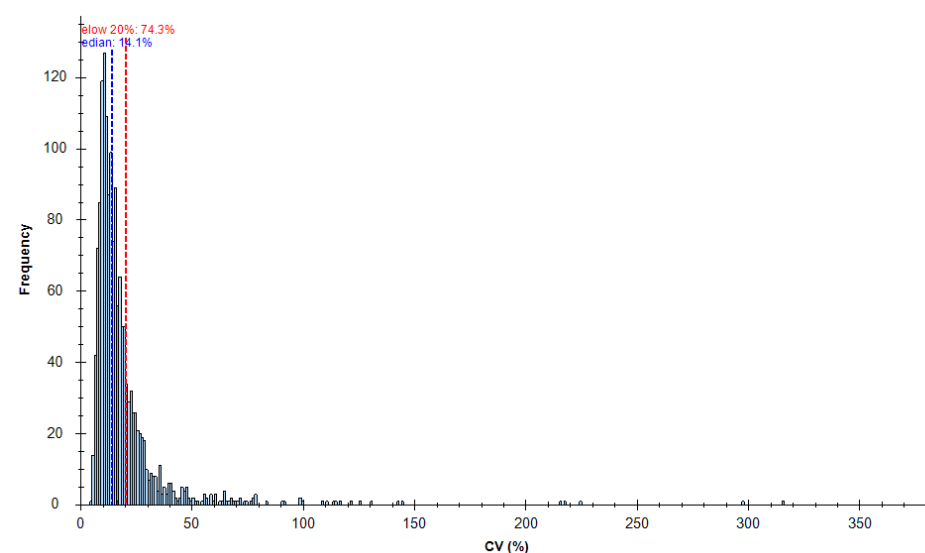


Figure 4. Peptide peak area %CV distribution based on MS1 scan. Using an equalization median normalization, the median peptide peak area %CV was 14.1% across 24 samples of 20 μL plasma.

Three-in-One Workflow Extracts Lipids with High Reproducibility and Broad Coverage

Table 3. Reproducibility of automated lipid extraction from 20 μ L plasma samples. %RSD for 11 deuterated lipids was calculated across 24 samples (%RSD, Total) and multiple injections of a pooled QC (%RSD, LC/MS), the difference between the two indicating the %RSD for sample prep using the Bravo Liquid Handler.

Analyte	%RSD, Total	%RSD, LC/MS	%RSD, Bravo
DG(15:0_18:1)d7	12.39	4.38	8.01
LPC(18:1)d7	10.96	3.06	7.90
LPE(18:1)d7	8.99	6.52	2.47
PC(15:0_18:1)d7	10.24	3.95	6.29
PC(P18:0_18:1)d9	12.02	2.25	9.76
PE(15:0_18:1)d7	13.46	3.91	9.56
PG(18:1)d7	11.63	3.42	8.21
PI(15:0_18:1)d7	9.95	5.02	4.92
PS(15:0_18:1)d7	11.44	4.62	6.82
SM(18:1_18:1)d7	11.06	5.30	5.76
TAG(48:1)[NL-18:1]d7	12.65	2.72	9.93

Targeted lipidomics analysis detected 615 lipids across commonly analyzed lipid classes, including ceramides, phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, sphingomyelins, and triacylglycerols. Preliminary percent recoveries were determined for two, 900 μ L 2:1 methanol:DCM elutions.

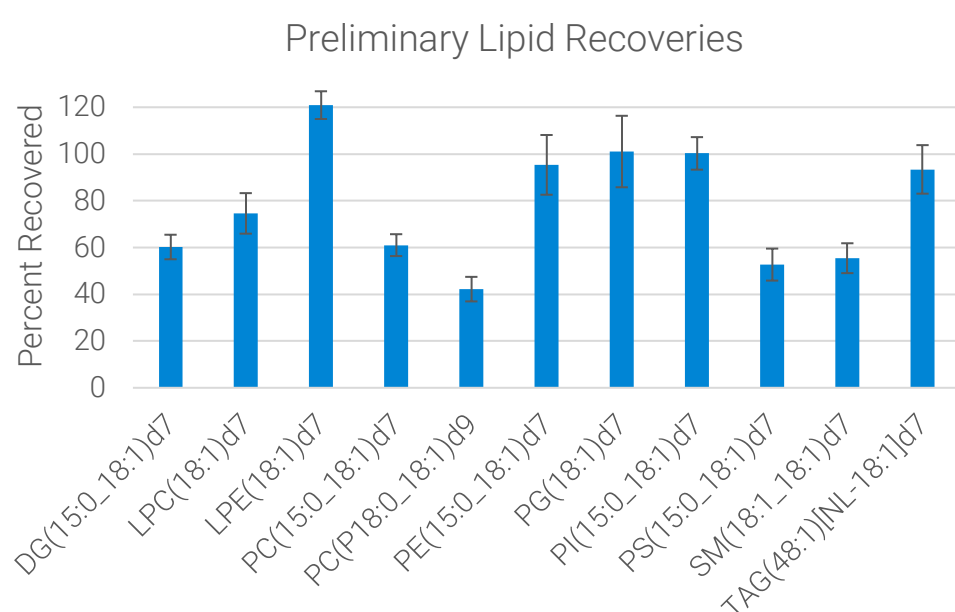


Figure 5. Preliminary recoveries for lipids extracted from mouse SPLASH spiked 20 μ L plasma samples. Recovery range was 40-120%, error bars represent SD. Based on a previous study,¹ we anticipate higher percent recoveries are possible with additional elution steps.

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Comparison of Lipid LC/MS Traces for 3 in 1 and 2 in 1 (Metabolite + Lipid) Workflows

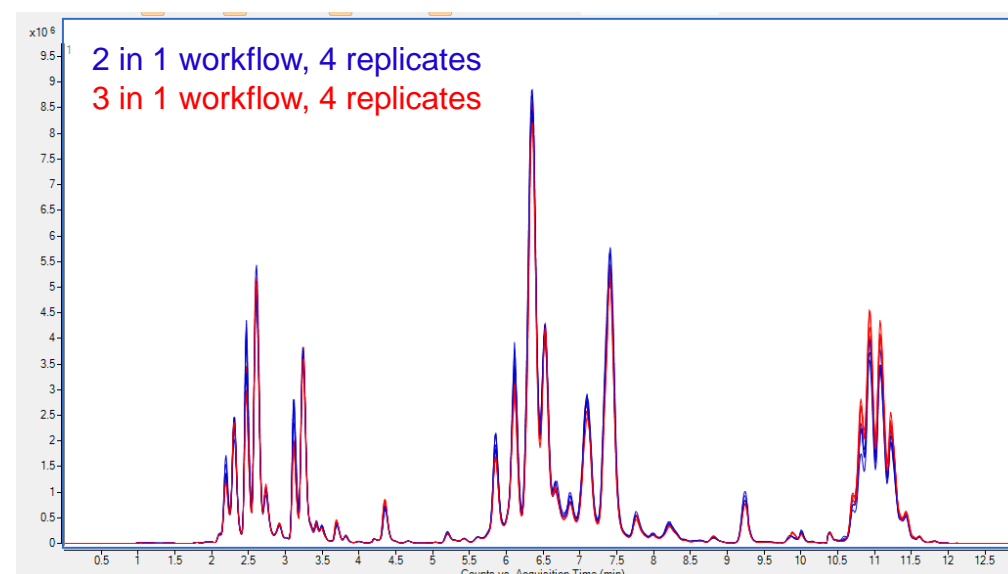


Figure 6. Replicate injections of lipids extracted using a 2 in 1 workflow¹ and the 3 in 1 workflow show consistent TICs and good overlap for both workflows.

Conclusions

- This comprehensive omics workflow offers great coverage and reproducibility for metabolites, lipids and proteins from small volumes of plasma.
- Easy-to-implement, tested sample prep protocol and LC/MS methods enable completion of a multi-omics study in weeks.
- Flexible workflow enables collection and analysis of any combination of 1, 2 or 3 sample fractions.

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