



Improving assay efficiency for blood-brain barrier (BBB) integrity biomarker quantitation in neurovascular disorder research using microflow LC-MS/MS

Dhaval Kumar Patel¹, Ehsan Nozohouri¹, Ulrich Bickel¹, Ebru Selen², Rahul Baghla² and Eshani Galermo²

¹Texas Tech University Health Sciences Center, USA and

²SCIEX, USA

Key benefits for analysis of sucrose biomarker using the SCIEX 7500 system coupled with the M5 microflow LC system

This technical note demonstrates a sensitive method for the quantitation of [¹³C₁₂]sucrose in Laser Micro Dissected (LMD) brain tissue samples using triple quadrupole mass spectrometry. Microflow LC-based separation indicated >5-fold improvement in signal-to-noise (S/N) compared to analytical flow conditions (**Figure 1**), enabling a lower limit of quantitation (LLOQ) of 50 pg/mL in LMD tissue samples.

The blood-brain barrier (BBB) plays a crucial role in maintaining the central nervous system's stability by preventing harmful substances entry into the brain. Dysfunction of the BBB has been linked to various neurological disorders. To investigate BBB integrity and assess tissue dysfunction or regulation precisely, a sensitive quantitation method is required to analyze extremely low levels of biomarkers in both whole brain tissue and LMD brain samples.

Here, a reliable and highly sensitive workflow is presented to support the quantitative analysis of [¹³C₁₂]sucrose using the SCIEX 7500 system coupled with the M5 microflow LC system.

- **Low pg/mL level of quantitation:** Achieve 50 pg/mL LLOQ for quantitation of [¹³C₁₂]sucrose for the analysis in LMD brain tissue samples
- **Enhanced sensitivity:** Achieve a 5-fold improvement in S/N using the M5 microflow LC system compared to conventional analytical flow conditions
- **Lower solvent consumption:** The microflow workflow uses up to 20x less solvent than the analytical flow method for the LC separation, resulting in significant cost savings, waste reduction, and a more sustainable solution
- **Robust analytical performance:** Achieve accurate quantitative performance with %CV <10% at all concentration levels across a linear dynamic range (LDR) of 4.3 orders of magnitude
- **Streamlined data management:** SCIEX OS software, a 21 CFR Part 11-compliant platform, simplifies data acquisition and processing

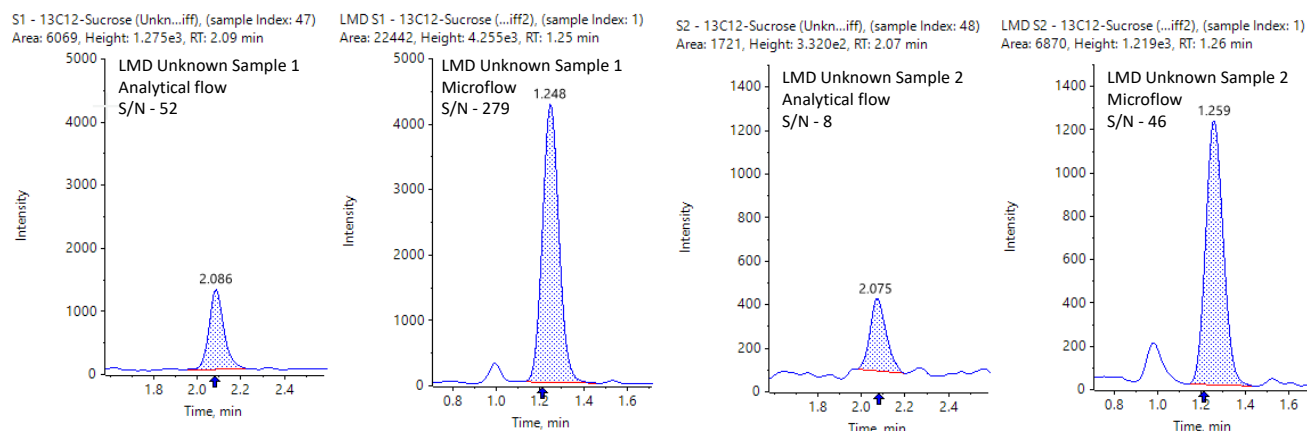


Figure 1: Representative extracted ion chromatograms (XICs) for [¹³C₁₂]sucrose in brain LMD samples. XICs from unknown sample 1 and sample 2 were analyzed using analytical flow conditions and microflow conditions, indicating a significant >5-fold improvement in S/N using microflow conditions compared to analytical flow conditions

Introduction

Quantifying the [$^{13}\text{C}_{12}$]sucrose marker in LMD tissues is of utmost importance as it allows us to investigate the location of BBB dysfunction or regulation. ^{13}C -labeled sucrose has been established as the most accurate marker of BBB integrity, and it is superior to previously used radioactive sucrose isotopes.¹ The current approach involves cutting larger LMD tissue samples to bring them within the quantitation range. However, this compromises the ability to study specific tissue locations accurately. Therefore, implementing advanced sensitivity-enhancing technologies will enable the mitigation of this limitation and unlock critical insights into BBB integrity at a microscopic level.

The successful enhancement of sensitivity for the [$^{13}\text{C}_{12}$]sucrose marker holds great potential to significantly advance our understanding of BBB integrity and permeability in neurological disorders.² By enabling precise spatial analyses within LMD tissues, this improvement will offer invaluable insights into disease mechanisms and potential therapeutic targets. Such advancements would significantly enhance the strength and scope of research, opening new avenues for cutting-edge research and potential breakthroughs in the field.

Methods

Sample preparation: The brain tissue samples were cut in 500,000 and 200,000 μm^2 sizes using a Laser Microdissection Microscope (Leica LMD6) and were homogenized with 80:20 (v/v) acetonitrile/water. [$^2\text{H}_2$]sucrose was used as the internal standard. An injection volume of 2 μL was used for analysis.³

Chromatography: Microflow chromatographic separation was performed on the M5 microflow LC system used in direct injection mode at a 10 $\mu\text{L}/\text{min}$ flow rate using a [Luna omega sugar](#) micro LC (3 μm , 0.3mm x 100mm) microflow LC column. The isocratic elution was performed using a mobile phase consisting of 0.1% v/v ammonium hydroxide in 60:40 (v/v) acetonitrile/water mixture.

Analytical flow chromatographic separation was performed on the Shimadzu LC-40 system at a flow rate of 0.2 mL/min using a Waters BEH amide (1.7 μm , 2.1mm x 50mm) column

in isocratic conditions with a mobile phase consisting of 0.1% v/v ammonium hydroxide in 72:28 (v/v) acetonitrile/water mixture.¹

Mass spectrometry: The optimized source and gas parameters are listed in **Table 2** and the MRM parameters are included in **Table 3**.

Table 2: Source and gas parameters.

Polarity	Microflow	Analytical flow
	Negative	
Ion source gas 1	30 psi	40 psi
Ion source gas 2	35 psi	50 psi
Curtain gas	40 psi	45 psi
Source temperature	400°C	400°C
Ion spray voltage	-4500 V	-2000 V
CAD gas	10	8

Table 3: MRM parameters used for quantitation.

ID	Precursor ion (m/z)	Fragment ion (m/z)	CE (V)	CXP (V)	Q0D (V)
[$^{13}\text{C}_{12}$]sucrose	353.1	91.994	-26	-13	-50
[$^2\text{H}_2$]sucrose (IS)	343.1	88.964	-25	-10	-50

Data processing: Data collection and analysis were performed using SCIEX OS software, version 3.3.1. Peaks were integrated using the MQ4 algorithm and a weighting of $1/x^2$ was used for quantitation.

Quantitative performance on the SCIEX 7500system

A calibration curve was analyzed for the concentrations ranging from 0.05 – 1000 ng/mL. Each concentration was analyzed in triplicate to evaluate reproducibility.

[¹³C₁₂]sucrose achieved an LLOQ of 50 pg/mL on the SCIEX 7500 system with the M5 microflow LC system (**Figure 2**).

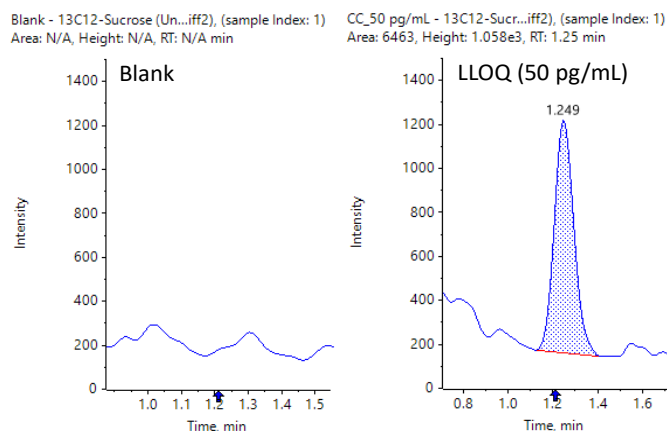


Figure 2: Representative XICs of the blank and LLOQ for the [¹³C₁₂]sucrose spiked at 50 pg/mL.

Linearity was achieved across a range of concentrations from 0.05 to 1000 ng/mL with a correlation of determination (r^2) of 0.997 for [¹³C₁₂]sucrose (**Figure 3**). An LDR of 4.3 orders of magnitude was reached.

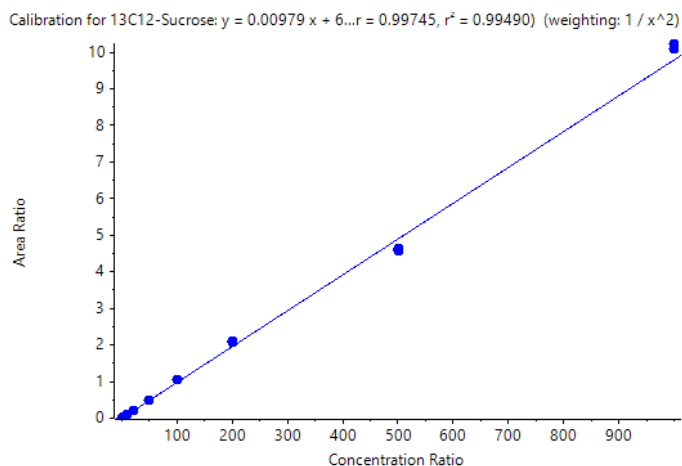


Figure 3: Calibration curve for the quantitation of [¹³C₁₂]sucrose. Linearity was established between 0.05 ng/mL and 1000 ng/mL, generating an LDR of >4 orders of magnitude with an r^2 of 0.995.

Analytical performance was evaluated based on the criteria that the accuracy of the calculated mean should be between 80% and 120% at the LLOQ and between 85% and 115% at the higher concentrations. In addition, the %CV of the calculated mean of the concentration should be <20% at the LLOQ and <15% at all higher concentrations.

The assay accuracy was within $\pm 13\%$ of the actual concentration and the %CV was <10%. Calculated percent accuracy and %CV values were within the acceptance criteria at each concentration level (**Figure 4**).

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Average Accuracy across Replicates
1	13C12-Sucrose	0.050	3 of 3	0.052	0.005	9.89	104.
2	13C12-Sucrose	0.200	3 of 3	0.175	0.003	1.85	87.6
3	13C12-Sucrose	0.500	3 of 3	0.454	0.016	3.64	90.7
4	13C12-Sucrose	1.000	3 of 3	0.993	0.019	1.89	99.3
5	13C12-Sucrose	2.000	3 of 3	1.914	0.019	0.993	95.7
6	13C12-Sucrose	5.000	3 of 3	5.014	0.063	1.25	100.
7	13C12-Sucrose	10.000	3 of 3	10.444	0.220	2.11	104.
8	13C12-Sucrose	20.000	3 of 3	20.742	0.334	1.61	104.
9	13C12-Sucrose	50.000	3 of 3	50.573	0.127	0.251	101.
10	13C12-Sucrose	100.000	3 of 3	107.943	0.262	0.242	108.
11	13C12-Sucrose	200.000	3 of 3	214.253	0.843	0.393	107.
12	13C12-Sucrose	500.000	3 of 3	470.258	3.782	0.804	94.1
13	13C12-Sucrose	1000.000	3 of 3	1039.286	8.363	0.805	104.

Figure 4: Quantitative performance for [¹³C₁₂]sucrose analysis. Reproducibility and accuracy results were determined from the calibration curve standards across 3 replicates at each concentration. Statistical results were summarized using the Analytics module in SCIEX OS software.

Enhanced sensitivity with M5 microflow LC system

The calibration curve and unknown brain LMD samples were analyzed under analytical flow conditions at 0.2 mL/min, and data was compared to the microflow conditions. The results indicated a 5-fold increase in S/N on average using the M5 microflow LC system, compared to conventional analytical flow conditions for both calibration curve samples and unknown brain LMD samples (**Figures 1 and 5**).

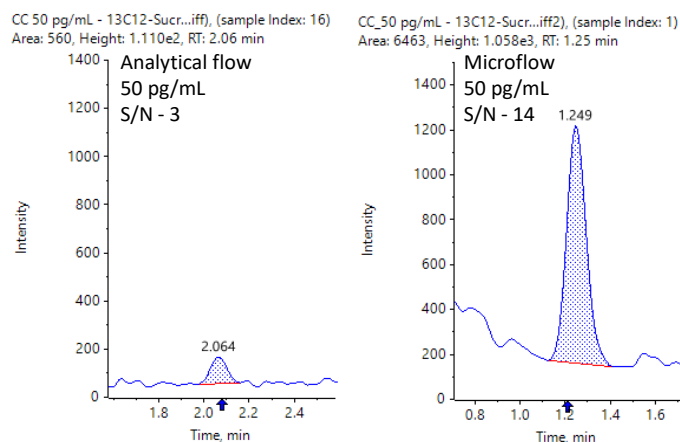


Figure 5: Representative XICs from analytical flow and microflow experiments for a calibration standard at 50 pg/mL. The left panel displays the XIC using analytical flow conditions while the right panel shows the XIC under microflow conditions, demonstrating a 5x improvement in S/N.


Compliance-ready SCIEX OS software

Equivalent SCIEX OS software capabilities for regulated bioanalysis can be executed on the SCIEX 7500 system, ensuring high fidelity when performing method transfers while retaining critical compliance features.

SCIEX OS software is a closed system and requires records and signatures to be stored electronically, meeting the regulations outlined by 21 CFR Part 11. SCIEX OS software can open raw data files from any visible storage location within a closed network by using designated processing

workstations. **Figure 6** illustrates the features of SCIEX OS software that are used to monitor the audit trail, acquire and process data, and configure user access.

The audit trail feature enables users to audit critical user actions and locks in data integrity. The Central Administrator Console (CAC) feature allows users to centralize acquisition and processing using a single platform to maximize efficiency for multi-instrument laboratories, independent of compliance standards. The configuration module allows users to assign roles and access as the administrator, method developer, analyst and reviewer.



Audit Trail

Easily search and filter for specific high-risk events in audit trail viewer. Built-in data integrity features allow you to tailor each functionality specifically to meet compliance needs and data security requirements.



Central Administration

Users can manage groups, role definitions, workstations and projects across all systems using the SCIEX OS software Central Administrator Console (CAC). It supports all regulated and non-regulated compliance standards.



Configuration

Assign users and access to administrator, method developer, analyst and reviewer roles under the audit trail module. Easily customize the role and specify level of access.



Filter Audit Trail

Find instances where

Sample Name: [dropdown] Is: [radio] Standard [dropdown]

Reason: [dropdown] Is: [radio] manual integration [dropdown]

<No Filter> [dropdown] Is: [radio] [dropdown]

And where time and date are:

From: 3/3/2023 12:00 AM To: 3/3/2023 12:00 AM

OK Cancel



Centralized Management

User Management
User Roles and Permissions
Workstation Management
Project Management
Workgroup Management

Manage Workgroups and Assignments

Method Developers

Reviewers

Users
Workstations
Projects

Use Central Settings for Projects

Assign or Unassign Project Root from Project Pool

Project Root Directory

Information: Local\Opt\Users\Local\CAC Demo

CAC Demo

Save Cancel

Confirm Change Events

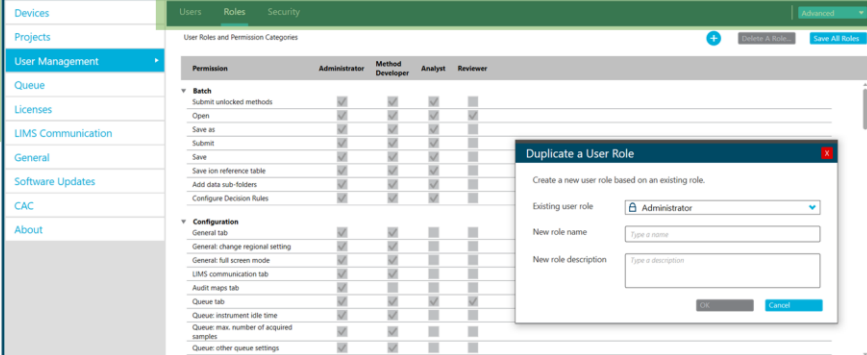
Change Events

Timestamp	Event Name	Event Description
3/3/2023 4:48:49 PM	Project settings enabled/disabled in a workgroup	Project based central settings enabled for Workgroup Reviewers
3/3/2023 4:48:49 PM	Project assigned/unassigned to a workgroup	Local\Users\Local\Opt\Users\Local\CAC Demo\Default assigned to Workgroup Reviewers

Signature: Timestamp: 3/3/2023 4:48:49 PM
User Name: ABCDEVELOP\Project
Full User Name: SLT Project3

Reason: Project assigned
Password: [password field]

Cancel Change Record Change



Users Roles Security

User Roles and Permission Categories

Permission	Administrator	Method Developer	Analyst	Reviewer
Batch				
Submit unlocked methods	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Open	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Save as	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Submit	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Save	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Save ion reference table	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Add data sub-system	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Configure Decision Rules	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Configuration				
General tab	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
General change regional settings	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
General full screen mode	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
LIMS communication tab	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Audit maps tab	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Queue tab	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Queue instrument idle time	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Queue max number of acquired samples	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Queue other queue settings	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Duplicate a User Role

Create a new user role based on an existing role.

Existing user role: Administrator

New role name: [text field]

New role description: [text field]

OK Cancel

Figure 6: Features of SCIEX OS software for monitoring user access and evaluating the audit trail. The audit trail view allows users to filter for high-risk events easily and enables data integrity features to meet compliance requirements. The software features a Central Administrator Console (CAC) to manage users and groups, role definitions, workstations and projects across all systems. The CAC feature supports both regulated and non-regulated compliance standards. The configuration module enables users to quickly set up roles and levels of access for the administrator, method developer, analyst and reviewer levels.

Conclusions

- An LLOQ of 50 pg/mL was achieved for the analysis of [¹³C₁₂]sucrose in mice brain samples
- Good linearity was accomplished across concentrations ranging from 0.050–1000 ng/mL with an r² of 0.995 and an LDR spanning 4.3 orders of magnitude
- Compared to conventional analytical flow conditions, the M5 microflow LC system reached a 5-fold improvement in S/N. The improvement in sensitivity could enable the investigation of the precise location of BBB dysfunction or regulation.
- Accurate and highly reproducible quantitative performance was demonstrated at all concentration levels with a %CV <10%
- The microflow method achieved up to 20x lower solvent consumption compared to an analytical flow method. Minimizing LC-MS-grade solvent consumption increases cost savings, reduces waste generation, and promotes an eco-friendly option.

- The assay exhibited the ability to routinely quantify low levels of [¹³C₁₂]sucrose with a 4-minute runtime, enabling bioanalytical labs to deliver high-quality data with good throughput
- Retain data management and compliance-readiness (21 CFR Part 11) features using SCIEX OS software to support non-regulated and regulated bioanalysis on the SCIEX 7500 system

References

1. Miah et. al. Evaluation of [¹⁴C] and [¹³C]Sucrose as Blood–Brain Barrier Permeability Markers, [Journal of Pharmaceutical Sciences, Volume 106, Issue 6, 2017.](#)
2. Kadry et al. A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity. [Fluids Barriers CNS. 2020;17\(1\):69.](#)
3. Noornai et. al. LC-MS/MS based in-vitro and in-vivo investigation of blood-brain barrier integrity by simultaneous quantitation of mannitol and sucrose. [Fluids Barrier CNS17, 61 \(2020\).](#)

The SCIEX clinical diagnostic portfolio is For In Vitro Diagnostic Use. Rx Only. Product(s) not available in all countries. For information on availability, please contact your local sales representative or refer to <https://sciex.com/diagnostics>. All other products are For Research Use Only. Not for use in Diagnostic Procedures.

Trademarks and/or registered trademarks mentioned herein, including associated logos, are the property of AB Sciex Pte. Ltd. or their respective owners in the United States and/or certain other countries (see www.sciex.com/trademarks).

© 2024 DH Tech. Dev. Pte. Ltd. MKT-30856-A



Headquarters

500 Old Connecticut Path | Framingham, MA 01701
USA
Phone 508-383-7700
sciex.com

International Sales

For our office locations please call the division headquarters or refer to our website at sciex.com/offices