Rapid Adenosine Bioanalysis with LS-LC-MS/MS

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Poster#: P81

Background

Adenosine is an important signaling molecule composed of adenine and nucleoside. In the hypoxic tumor microenvironment (TME), highly expressed CD73 catalyzes AMP dephosphorylation to form large amounts of adenosine. It can inhibit immune cell activity, enhance immune escape, and promote tumor growth. The traditional LC-MS method for adenosine analysis is complicated, often requiring sample derivatization[1], complex mobile phases (such as in HILIC mode), or lengthy analytical conditions[2].This limits its application for research, particularly in studies with large numbers of samples.

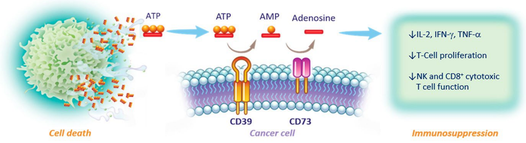


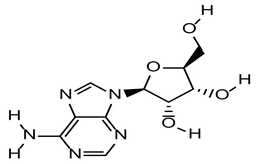
Figure 1. Conversion of extracellular ATP to adenosine by CD39 and CD73 [3]

Objective

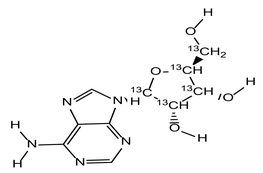
To develop a rapid and direct method of adenosine in biological samples using high-speed autosampler (Leadsampler-1) and multiplex LC system, thereby aiding scientists in understanding the role adenosine plays in the TME and its relationship to cancer.

Method

Compound information



Adenosine
Chemical formula: $C_{10}H_{13}N_5O_4$
Exact mass: 267.10



$^{13}C_5$-Adenosine
Chemical formula: $C_5{}^{13}C_5H_{13}N_5O_4$
Exact mass: 272.11

Sample preparation

The reaction mixtures contain CD73 enzyme and adenosine was incubated. Then samples were mixed with water acidified by trichloroacetic acid (TCA) containing $^{13}C_5$-adenosine as internal standard (IS), vortexed well and centrifuged. Aliquots of 150 µL supernatant were transferred into a 96-well plate and then analyzed on a high-throughput LC-MS/MS system.

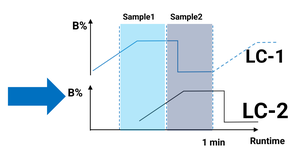
High-throughput LC-MS/MS system

Shimadzu Nexera UHPLC-40

LS-I Autosampler

SCIEX Triple Quad™ 6500+ MS System

LeadScape™ software & Analyst® software



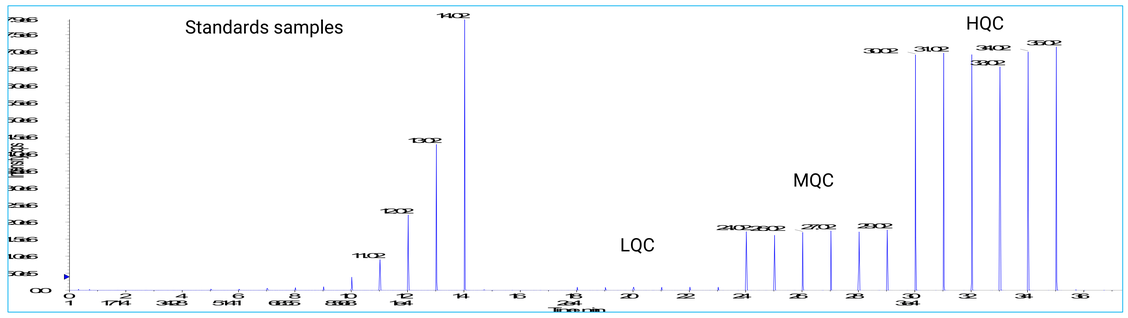
Multiplex Stagger Injection Mode

Analytical condition

LC System	Shimadzu Nexera UHPLC-40
MS System	SCIEX Triple Quad™ 6500+
Mobile Phase	A: 0.1% formic acid in water; B: 0.1% formic acid in acetonitrile
Column	Waters ACQUITY UPLC C18 Column
Run Time	0.5 min per injection
MS Transition	Adenosine (m/z=268.0/136.0); $^{13}C_5$-adenosine (IS) (m/z=273.2/136.2)

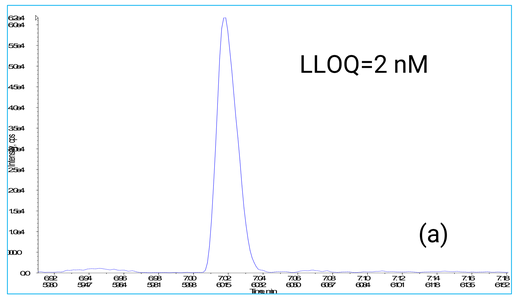
Results

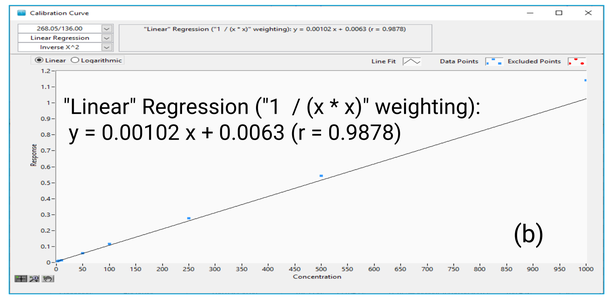
Typical chromatogram of adenosine



By using TCA, adenosine exhibited a retained symmetrical peak under the reversed-phase chromatography system.

Sensitivity(a) and linearity(b)





Within-run precision and accuracy results for adenosine

Analytical Run ID	LQC (6 nM)	%Bias	MQC (200 nM)	%Bias	HQC (800 nM)	%Bias
Adenosine	6.36	5.94	207.93	3.97	894.22	11.78
	5.07	-15.44	198.31	-0.85	883.61	10.45
	6.08	1.34	207.38	3.69	877.59	9.70
	5.80	-3.38	208.81	4.41	866.54	8.32
	6.19	3.16	197.74	-1.13	891.54	11.44
	6.09	1.48	218.27	9.14	902.99	12.87
Within-run Mean	5.93		206.41		886.08	
Within-run SD	0.46		7.62		12.97	
Within-run %CV*	7.72		3.69		1.46	
Within-run %Bias**	-1.15		3.20		10.76	

*Within-run %CV must be less than or equal 20%. **Within-run %Bias must be within ±25%.

Conclusion

- A high-throughput LS-I-LC-MS/MS method was established first for quantitative analysis of adenosine, good peak shape and an LLOQ of 2 nM were achieved.
- Linearity, within-run accuracy and precision were evaluated. The results demonstrated the reliability and reproducibility of the method.
- The analytical time was shortened to 0.5 min/sample by the LS-I-LC-MS/MS system, which reduced 88% of the analysis time compared to literature method.

References

[1] Goodwin KJ, Gangl E, Sarkar U, Pop-Damkov P, Jones N, Borodovsky A, Woessner R, Fretland AJ. Development of a quantification method for adenosine in tumors by LC-MS/MS with dansyl chloride derivatization. Analytical biochemistry. 2019 Mar 1;568:78-88.

[2] Van Dycke A, Verstraete A, Pil K, Raedt R, Vonck K, Boison D, Boon P. Quantitative analysis of adenosine using liquid chromatography/atmospheric pressure chemical ionization-tandem mass spectrometry (LC/APCI-MS/MS). Journal of Chromatography B. 2010 Jun 1;878(19):1493-8.

[3] Jeffrey JL, Lawson KV, Powers JP. Targeting metabolism of extracellular nucleotides via inhibition of ectonucleotidases CD73 and CD39. Journal of Medicinal Chemistry. 2020 Aug 13;63(22):13444-65.

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