

# Application News

## No. C90

### Liquid Chromatography Mass Spectrometry

## Measurement of Adenosine Deaminase Activity in Urine with LCMS-8040

Adenosine deaminase (ADA) is an enzyme involved in the metabolism of nucleic acid within the cell, and converts the nucleic acid bases adenosine (ADO) and deoxyadenosine (dADO) into inosine and deoxyinosine, respectively (Fig. 1). We analyzed for adenosine and deoxyadenosine as indicators in checking for ADA enzyme activity.

Here we describe an example analysis performed using an LCMS-8040 high-performance liquid chromatograph-triple quadrupole mass spectrometer and employing an analytical protocol used by the Mass Spectrometry, Clinical Chemistry and Pharmacology Lab. of Meyer Children's Hospital (Florence, Italy).

### Sample Preparation and Analytical Conditions

Samples for analysis were extracted from urine in accordance with the preparation method shown in Fig. 2. Samples extracted from plasma and a dried blood spot (DBS) can also be analyzed, and the relevant preparative methods are shown in Fig. 2 for reference. LC and MS conditions are shown in Table 1. Multiple reaction monitoring (MRM) was performed with adenosine and deoxyadenosine as the target compounds and using <sup>13</sup>C-adenosine and <sup>13</sup>C<sub>5</sub>-deoxyadenosine as internal standards.

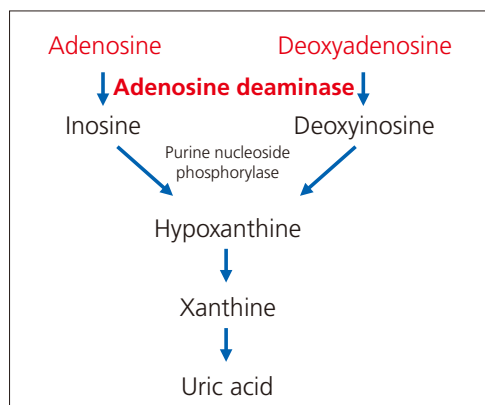


Fig. 1 Metabolic Pathway

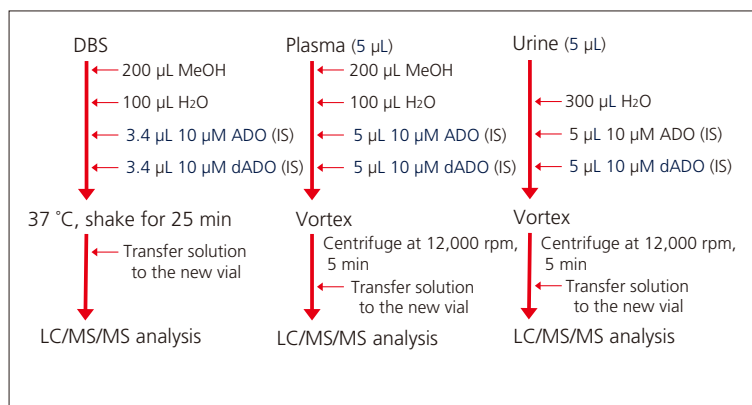


Fig. 2 Preparation Protocol

Table 1 Analytical Conditions

Column	: Synergi fusion RP (150 mm L. × 2 mm I.D., 4 µm)	Ionization Mode	: ESI(+)
Mobile Phase A	: 0.1 % HCOOH-H <sub>2</sub> O	Probe Voltage	: +4.5 kV
Mobile Phase B	: 0.1 % HCOOH-CH <sub>3</sub> CN	Nebulizing Gas Flow	: 3.0 L/min
Ratio	: 60 %B	Drying Gas Flow	: 15.0 L/min
Flowrate	: 0.2 mL/min	DL Temperature	: 200 °C
Column Temperature	: 30 °C	Block Heater Temperature	: 400 °C
Injection Volume	: 3 µL	MRM	: Adenosine (267.80>136.05) Deoxyadenosine (251.80>136.05) Adenosine IS (268.80>136.05) Deoxyadenosine IS (256.80>136.05)
Analysis Time	: 5 min		
MS	: LCMS-8040		

■ Analysis Results

Results of analysis are shown in Fig. 3. The "Sample" plot shows when there is no ADA enzyme activity in the sample, and the "Control" plot shows when ADA enzyme activity is present in the sample. A peak representative of deoxyadenosine was detected in the

"Sample" plot results, and no deoxyadenosine peak was detected in the "Control" plot results. This analytical system can be used to check for enzyme activity.

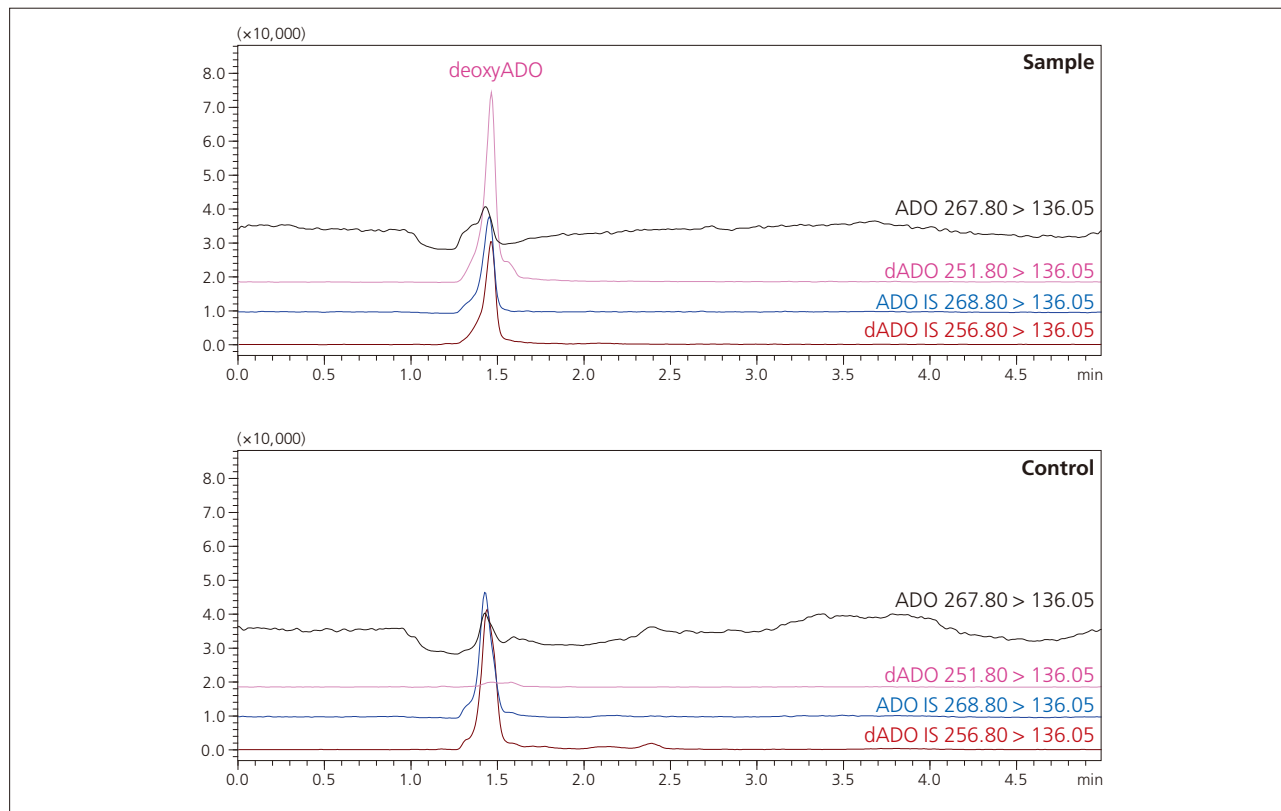


Fig. 3 Extracted-Ion Chromatograms of Target Compounds

[References]

- G la Marca et al. The inclusion of ADA-SCID in expanded newborn screening by tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* 88 (2014) 201-206  
 G la Marca et al. Tandem mass spectrometry, but not T-cell receptor excision circle analysis, identifies newborns with late-onset adenosine deaminase deficiency. *J ALLERGY CLIN IMMUNOL VOLUME 131, NUMBER 6 (2013) 1604-1610*

[Acknowledgement]

The present Application News was prepared with the assistance of materials and guidance provided by Dr. G. la Marca (Mass Spectrometry, Clinical Chemistry and Pharmacology Lab., Meyer Children's Hospital, Florence, Italy). We are sincerely grateful for his assistance.

Note: This analytical system may only be used for research applications, and may not be used for clinical diagnosis.