SHIMADZU APPLICATION NEWS
LAAN-A-LM-E002
● LIQUID CHROMATOGRAPHY MASS SPECTROMETRY
No.C37

LC-MS Analysis of Compounds Related to Nucleic Acids

Nucleic acids (DNA and RNA) are macromolecules polymerized from nucleotides that are composed of purine and pyrimidine bases, sugars and phosphate. Nucleic acids are vital components that carry the genetic information in living organisms. Other nucleic acid-related compounds such as bases, nucleosides and nucleotides in their free state are involved in the expression of various kinds of biochemical functions such as biosynthesis and metabolism. The level of these substances are maintained constant in individual organisms.

Purines are nucleic acid components with the purine ring. They are metabolized in the body, transformed to xanthine, further to uric acid, and a portion of it is excreted in the urine. Normally, the concentration of uric acid in the blood is kept constant. However, if the uric acid concentration increases abnormally under specific conditions (hyperuricemia), uric acid crystals can accumulate in the joints, causing gout. Nucleic acid components are also involved in various other metabolic abnormalities, and, therefore, nucleic acid components in biological samples such as blood and urine are being analyzed.

Nucleobases and nucleosides are generally separated by ion-exchange or reversed-phase HPLC and then detected by UV detectors. Here, we would like to introduce examples of the analysis of nucleic acid-related compounds using LC-MS featuring high sensitivity and the capability of obtaining molecular weight information.

Fig.1 shows the structural formulas and ESI mass spectra for one of the purine bases, adenine, and for the nucleoside adenosine. Under acidic conditions in the positive ion mode, the protonated molecule $(M+H)^+$ can be observed as the reference peak for both of them. Fig.2 shows the LC-MS analytical results for a mixture of nucleic acid standards. A Selected ion monitoring was conducted using the $(M+H)^+$ of each component as the detection ion. A satisfactory separation was obtained for 15 components.

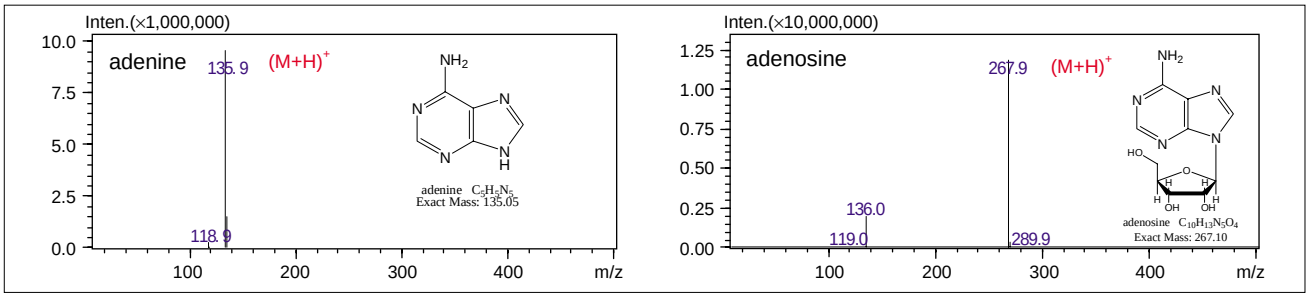


Fig.1 Positive ESI-MS Spectra of Adenine and Adenosine

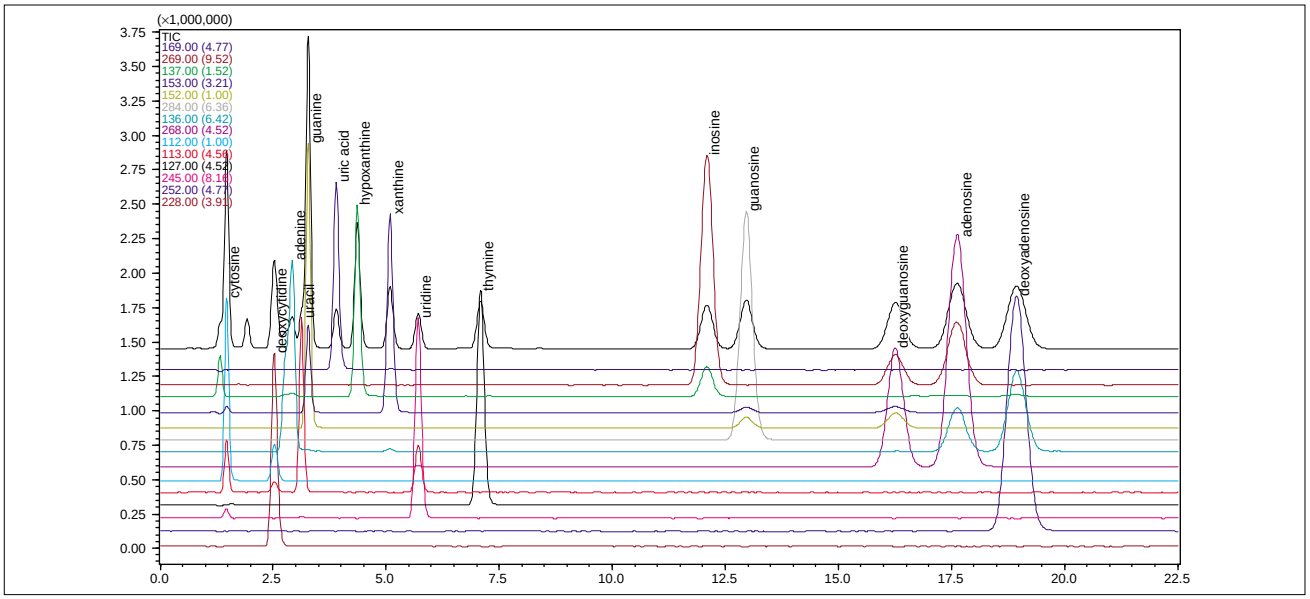
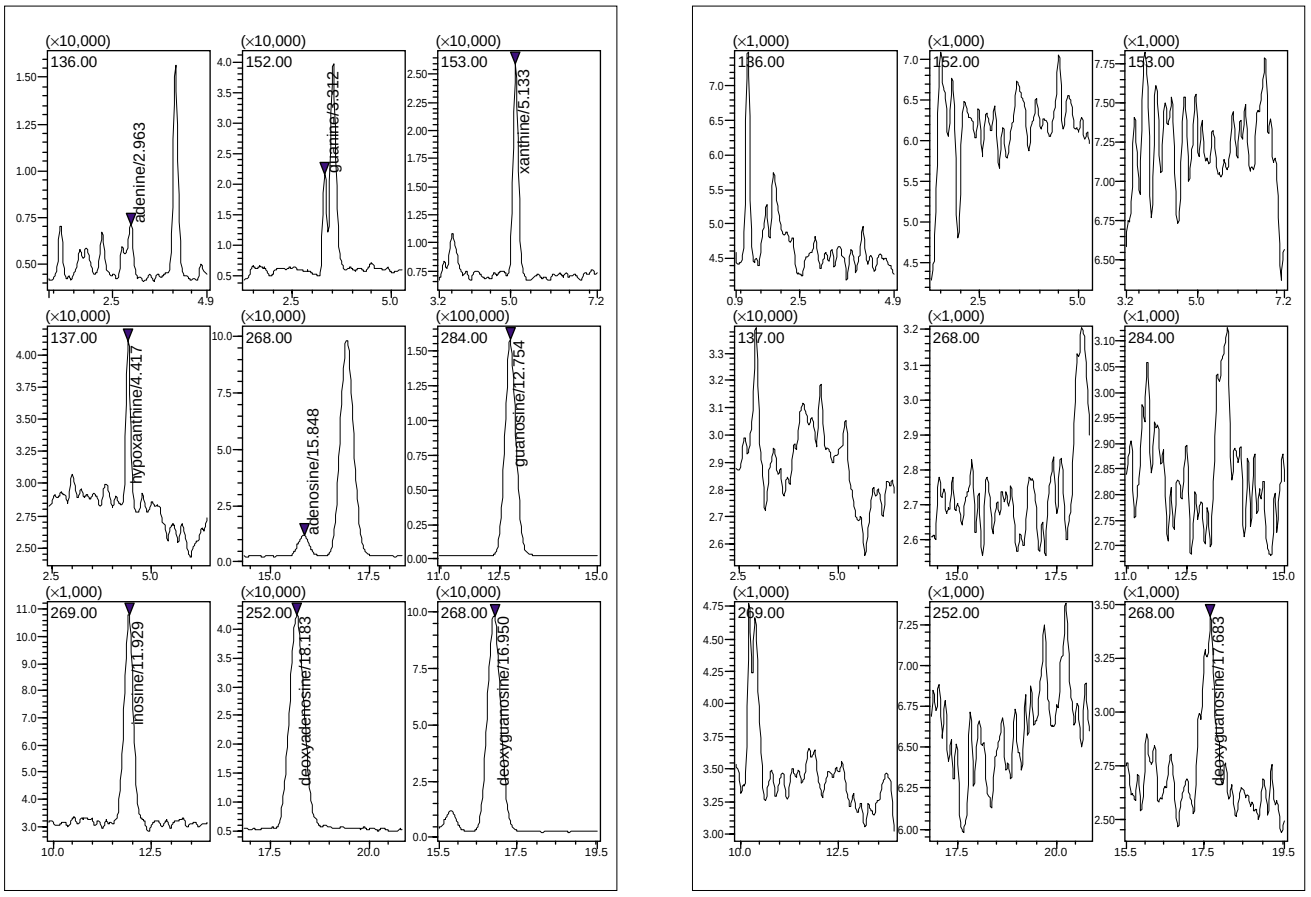


Fig.2 SIM Chromatogram of Mixture of Nucleo bases and Nucleosides

The causes of the increase in uric acid contained in the blood, which may lead to gout as mentioned above, includes genetic factors, diet and stress. Purines in food can also affect the level of uric acid in serum. An increasing concern is given to the content of purines in food, and products with reduced purine contents are now being marketed. Fig.3 shows analysis examples of purine bases and nucleosides contained in beer. The beer samples were diluted 100-fold with ultrapure water, filtrated and then analyzed. Xanthine and guanosine were detected in the ordinary beer (Fig.3A), while they were not detected in the low-purine beer (Fig.3B).



A: Beer　　B: Purine-cut low-malt beer

Fig.3 SIM Chromatograms of Purine Bases and Nucleosides in Beer (A) and Low-purine Low-malt Beer (B)

Table 1 Analytical Conditions

Column	: L-column ODS(150mmL. × 2.1mm I.D.)	
Mobile phase A	: 0.1% acetic acid–water	
Mobile phase B	: acetonitrile	
Time program	: 1%B(0-20min)–80%B(20.1-30min)	
Flow rate	: 0.2mL/min	
Injection volume	: 3μL	Column temperature : 30˚C
Probe voltage	: +4.5kV(ESI-Positive mode)	
CDL temperature	: 200˚C	Block heater temperature : 200˚C
Nebulizing gas flow	: 1.5L/min	
Drying gas pressure	: 0.1MPa	
CDL voltage	: +15V	
Q-array DC voltage	: Scan-mode	Q-array RF voltage : Scan-mode
Mass number of monitor ion	: m/z 136, 137, 152, 153, 169, 228, 252, 268, 269, 284	

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax. 81(3)3219-5710

Cable Add.:SHIMADZU TOKYO

Printed in Japan 3100-06501-10A-IK