SHIMADZU APPLICATION NEWS

LAAN-A-LM-E007

● LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

No.C43A

Analysis of Steroidal Anti-Inflammatory Drugs Using LC/MS

Although steroidal anti-inflammatory drugs (adrenal cortical hormones) have a very high anti-inflammatory efficacy, they are also known to have some side effects and it can be difficult to adjust their dosage to an appropriate level. There are also reports that steroidal anti-inflammatory drugs have been detected in imported products labeled as “health foods” and it is therefore important to monitor these drugs. An example of the analysis of steroidal anti-inflammatory drugs performed using LC/MS is presented here.

When determining the LC/MS analytical conditions, it is necessary to consider both ionization (detection) and chromatographic separation. Because steroidal anti-inflammatory drugs have a low polarity, positive ion atmospheric pressure chemical ionization (APCI-Positive) was used. In general, using a methanol mobile phase in APCI gives a greater ionization efficiency than an acetonitrile mobile phase. In this analysis, however, an acetonitrile mobile phase was selected in order to improve the separation between dexamethasone (peak ②) and betamethasone (peak ③). Usually, it is said that 0.2 mL/min is an appropriate mobile phase flow rate for a column with an inner diameter of 2 mm. In this case, however, a mobile phase flow rate of 0.3 mL/min was used in order to reduce the analysis time. Even under these conditions, the column head pressure was 7.8 MPa. Fig.2 shows the SIM chromatograms obtained for steroidal anti-inflammatory drugs.

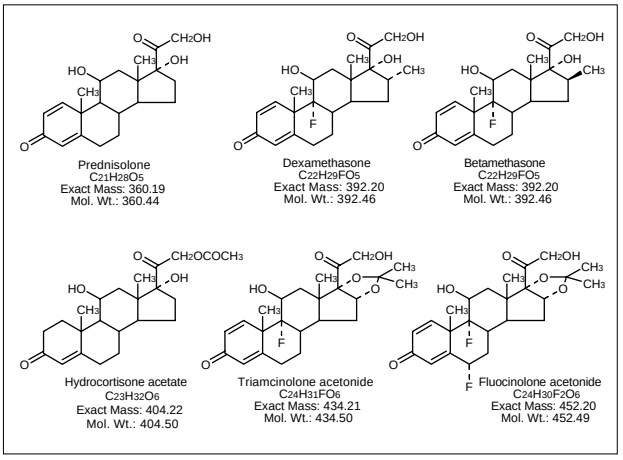

Fig.1 Structures of Six Steroidal Anti-Inflammatory Drugs

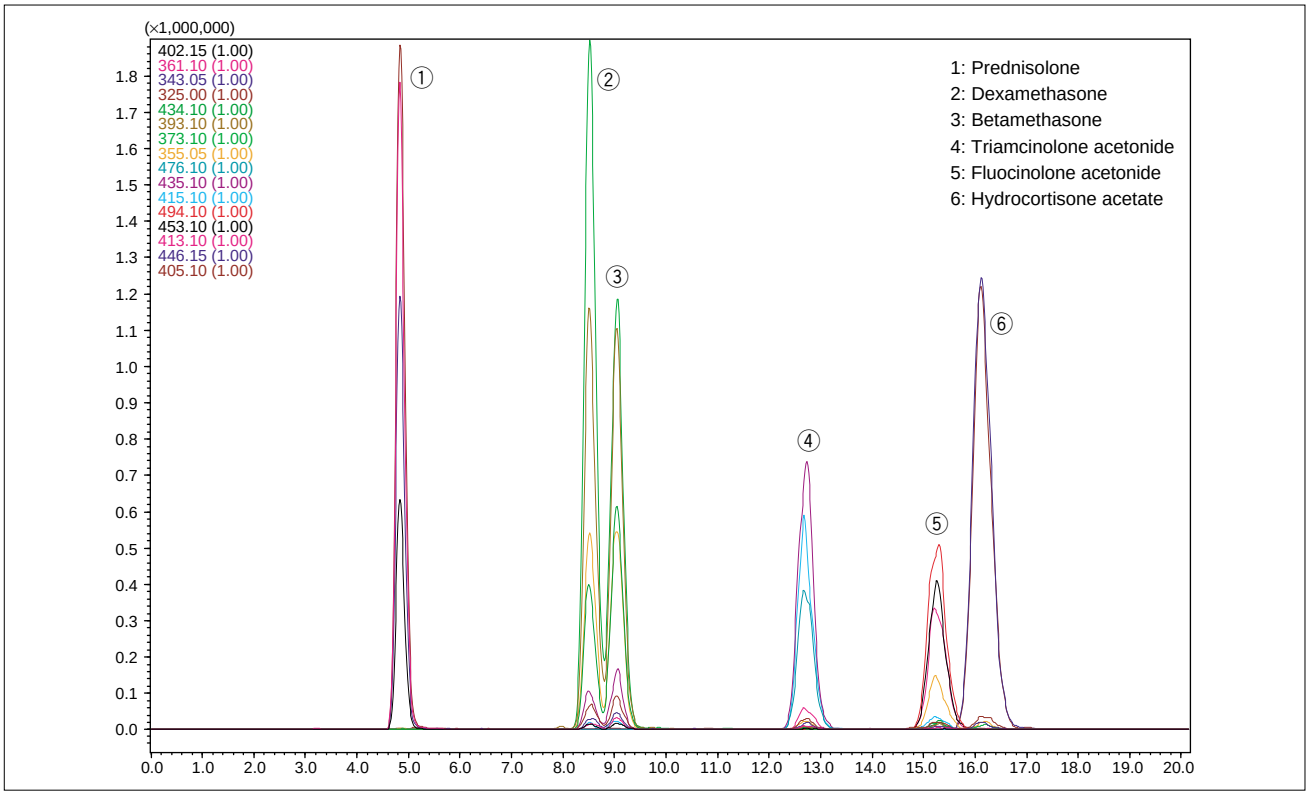

Fig.2 SIM Chromatograms for Six Steroidal Anti-Inflammatory Drugs

In these mass spectra, in addition to protonated molecules ($[M+H]^+$), fragment ions ($[M+H-H_2O]^+$ and $[M+H-2\times H2O]^+$), and mobile phase adduct protonated molecules ($[M+H+CH_3CN]^+$) are observed. In this analysis, the ions were all monitored (Table 1), the underlined protonated molecules were used as quantitative ions, and the other ions were used as reference ions. Fig.3 shows the calibration curves obtained by performing measurement 5 times at 6 concentrations in the range 6.4 to 2,000 ng/mL. Linearity ($r^2 > 0.9999$) was attained over a relatively wide range.

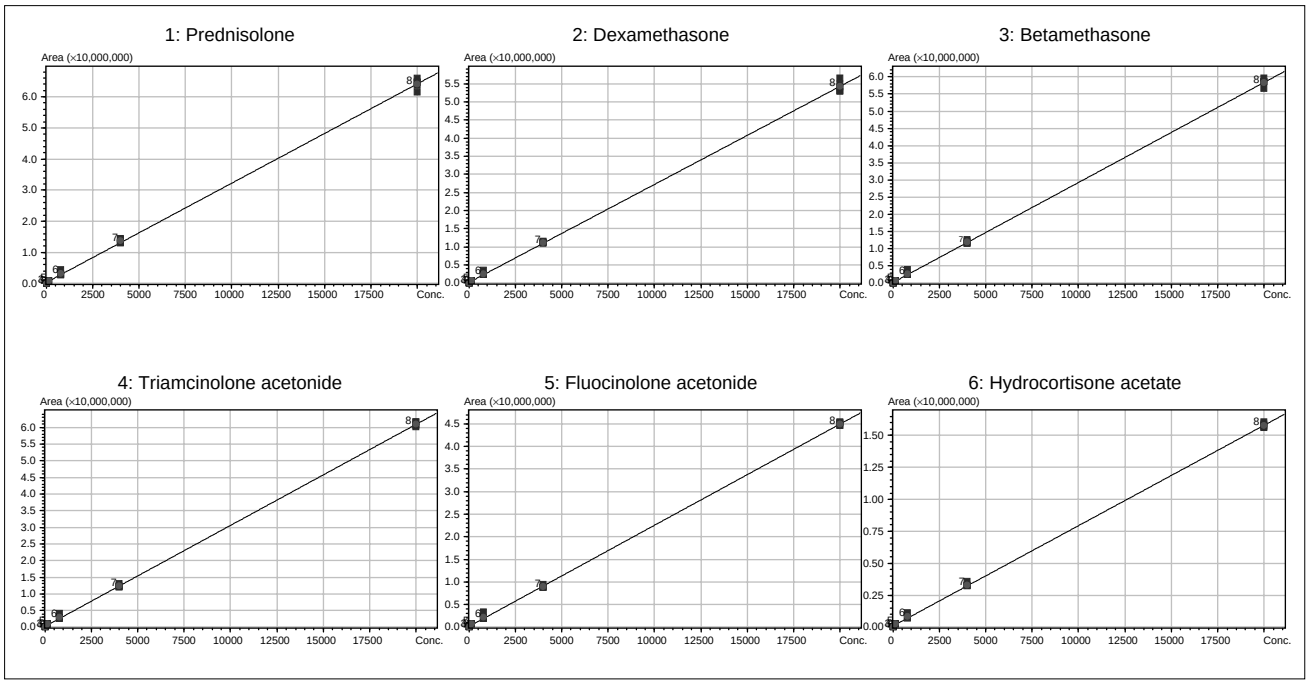

Fig.3 Calibration Curves for Six Steroidal Anti-Inflammatory Drugs (6.4 to 2,000 ng/mL) Area

Table 1 Analytical Conditions

Column	: Phenomenex Synergi MAX-RP (2.0 mm I.D. × 150 mmL.)
Mobile phase	: 0.1% formic acid-water /acetonitrile (70:30)
Flow rate	: 0.3 mL/min
Column oven temperature	: 40 ˚C
Injection volume	: 2 µL
Probe voltage	:+4.5 kV (APCI-Positive mode)
Nebulizer gas flow	: 2.5 L/min
Drying gas pressure	: 0.04 MPa
Probe temperature	: 350 ˚C
CDL temperature	: 200 ˚C
Block heater temperature	: 200 ˚C
CDL & Q-array voltage	: using default values
Interval	: 0.8 sec/ 16 chs
Mass number of monitor ions	: m/z 402.1, 361.1, 343.1, 325.1 for prednisolone
	m/z 434.1, 393.1, 373.1, 355.1 for betamethasone & dexamethasone
	m/z 476.1, 435.1, 415.1 for triamcinolone acetonide
	m/z 494.1, 453.1, 413.1 for fluocinolone acetonide
	m/z 446.1, 405.1 for hydrocortisone acetate

NOTES:
✻This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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