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Application News

No.C98

Liquid Chromatography Mass Spectrometry

Analysis of Steroids and NSAIDs Using the Shimadzu LCMS-8050 Triple Quadrupole Mass Spectrometer

With performance enhancing drug use considered contrary to fair play, along with the adverse effects they may have on the health and social welfare of athletes, sports doping testing is increasing and has been conducted according to the provisions of WADA (World Anti-Doping Agency).
Drugs that are registered as prohibited substances mainly fall into the categories of anabolic steroids (AAS) used primarily for building muscle strength, steroidal anti-inflammatory drugs for their anti-inflammatory and immunosuppressive effects, and narcotic and designer drugs. Also, non-steroidal anti-inflammatory drugs (NSAIDs) are drugs used to treat pain and inflammation as well as fever, and although they are not specified as prohibited drugs, their abuse by athletes is being viewed as a problem due to their side effects.
Since doping tests provide information for making critical decisions that actually affect athletes' lives, accuracy at the time of testing, as well fairness, are necessary. In this Application News, we introduce an accurate identification method for typical steroidal and non-steroidal anti-inflammatory drugs using multiple reference ion ratios, in addition to an example of high-sensitivity measurement.

MRM Analysis of Standards and Matrix-Matched Calibration Curves

We conducted MRM measurement of a mixed standard solution consisting of 14 typical steroids and non-steroidal anti-inflammatory drugs. Fig. 1 shows the MRM chromatograms obtained using the mixed standard solution (each component at 50 ng/mL), and Fig. 2 shows MRM chromatograms obtained from analysis of typical compounds at concentrations near their respective LOQs. Table 1 shows minimum and maximum concentrations used for generating the respective calibration curves. The lower limits of quantitation ranged from 10 to 100 pg/mL (20 – 200 fg on column), and excellent linearity was obtained over a wide range of more than 3 orders of magnitude for each substance.

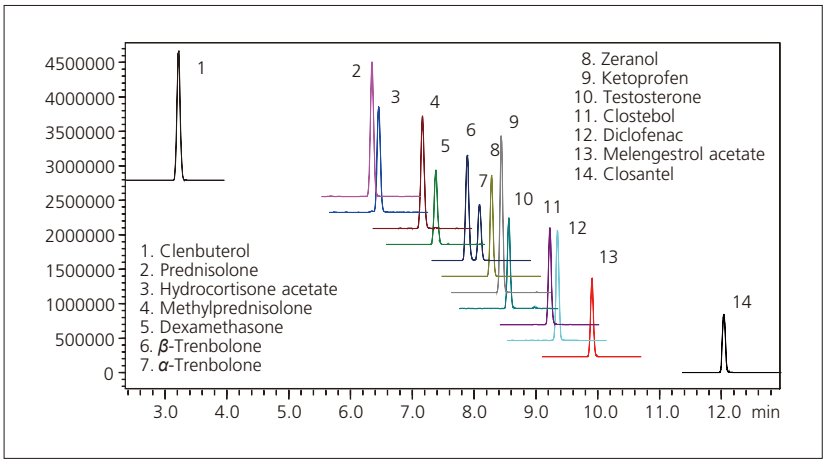


Fig. 1 Chromatograms of Steroids and NSAIDs

Table 1 Calibration Curve Min. /Max. Concentrations

Compounds	Min. Conc.	Max. Conc.
Clenbuterol	0.01	10
Prednisolone	0.05	20
Hydrocortisone acetate	0.1	50
Methylprednisolone	0.5	50
Dexamethasone	0.5	50
α-, β-Trenbolone	0.1	50
Zeranol	0.1	50
Ketoprofen	0.05	50
Testosterone	0.05	10
Clostebol	0.05	50
Diclofenac	0.01	50
Melengestrol acetate	0.05	50
Closantel	0.01	10

(Unit: ng/mL)

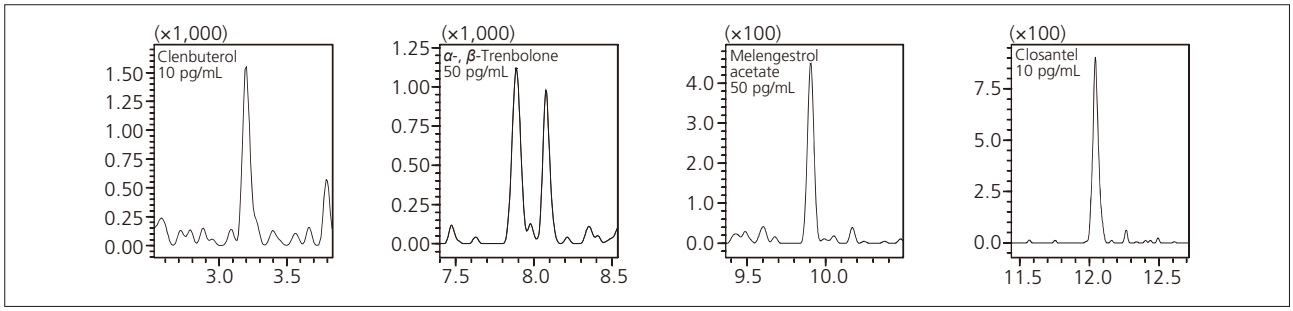


Fig. 2 MRM Chromatograms Near the LOQ of Typical Compounds

Peak Determination Using Multiple Reference Ions

When using multiple reference ions to conduct high-accuracy identification, the process of selecting and making the associated entries becomes complicated.
As of Labsolutions Ver. 5.65, however, this selection and entry process for qualifier MRM transitions now provides for automatic selection and entry as reference ions.

< Examples of New Features >

(1) Multiple reference ions are automatically entered (desired transitions can be selected and changed using drop-down menu).
(2) The ion ratio of the STD is automatically set as the reference value.
(3) A different allowable width of relative ion ratio can be set for each reference ion.
(4) The identification range (%) is automatically calculated from the ion ratio, allowable width and reference ion mode.

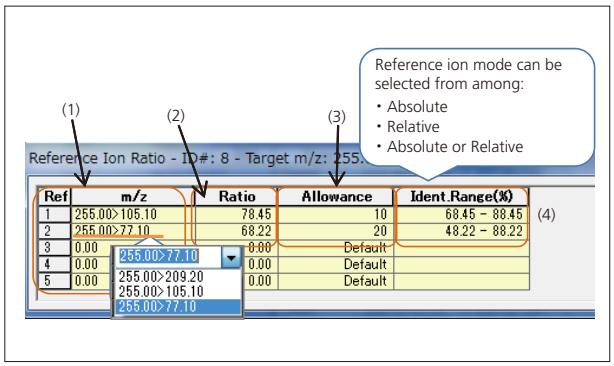
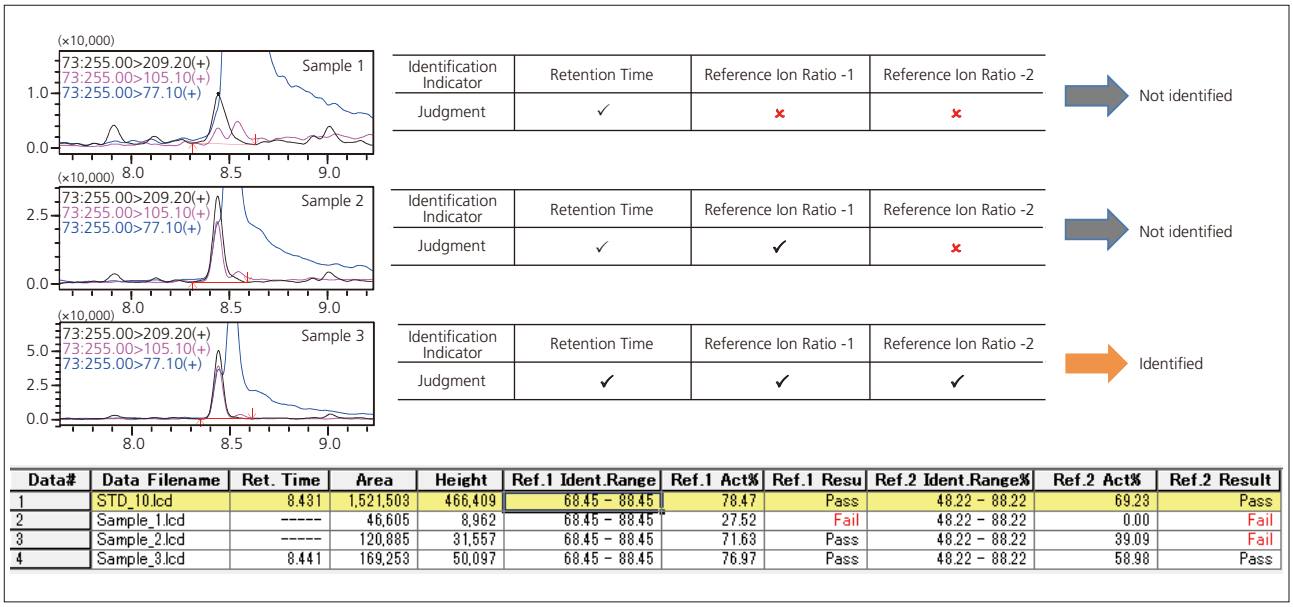


Fig. 3 Reference Ion Setting Window



Data#	Data Filename	Ret. Time	Area	Height	Ref.1 Ident.Range	Ref.1 Act%	Ref.1 Resu	Ref.2 Ident.Range%	Ref.2 Act%	Ref.2 Result
1	STD_10.lcd	8.431	1,521,503	466,409	68.45 – 88.45	78.47	Pass	48.22 – 88.22	69.23	Pass
2	Sample_1.lcd	-----	46,605	8,962	68.45 – 88.45	27.52	Fail	48.22 – 88.22	0.00	Fail
3	Sample_2.lcd	-----	120,885	31,557	68.45 – 88.45	71.63	Pass	48.22 – 88.22	39.09	Fail
4	Sample_3.lcd	8.441	169,253	50,097	68.45 – 88.45	76.97	Pass	48.22 – 88.22	58.98	Pass

Fig. 4 Example of Peak Determination Using Multiple Reference Ions

Table 2 Analytical Conditions

Column	: Shim-pack XR-ODS Ⅱ (2.0 × 75 mm, 2.2 µm)
Mobile Phase A	: 0.1 % Formic acid – Water
Mobile Phase B	: Acetonitrile
Time Program	: 1 %B (0 min) → 15 %B (1 min) → 40 %B (6 min) → 100 %B (10 - 13 min) → 1 %B (13.01 - 16 min) (12.01 - 15 min)
Flowrate	: 0.2 mL/min.
Injection Volume	: 2 µL
Oven Temperature	: 40 °C
Ionization Mode	: ESI (Positive / Negative)
Probe Voltage	: +4.5 kV / -3.5 kV
Neburizing Gas Flow	: 3.0 L/min.
Drying Gas Flow	: 10.0 L/min.
Heating Gas Flow	: 10.0 L/min.
Interface Temperature	: 400 °C
DL Temperature	: 200 °C
Block Heater Temperature	: 400 °C

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Related Products Some products may be updated to newer models.

LCMS-8050

High Performance Liquid Chromatograph Triple Quadr···

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