Application No. L3052

# (Glucocorticoid)

Glucocorticoids are steroid hormones produced in the cells of the fasciculus zona adrenal cortex, including cortisol (hydrocortisone) and cortisone. They promote gluconeogenesis in the liver, which raises blood sugar levels, and suppress tissue inflammatory reactions caused by trauma, infection, rheumatism, and other conditions.
Synthetic steroids, such as prednisolone, dexamethasone, and betamethasone, are commonly used clinically. In our application (L3047), we aimed to improve the detection sensitivity of steroids by using FIA-MS analysis with ammonia eluent. This time, we analyzed five different types of steroids using both ammonia and formic acid eluents.

Key words : L-column3 ammonia eluent LC-MS/MS steroid glucocorticoid

Column : USP category: L1

[ Analytical conditions ]

| | | |
|---|---|---|
| Column | : | *L-column3 C18* (2 µm, 12 nm); 3.0 mm I.D. × 100 mm L.; Cat. No. 823330 |
| Eluent | : | A: 5 mmol/L $NH_3$ in $H_2O$ or 0.1% HCOOH in $H_2O$ ; B:$CH_3CN$<br>A/B, 90/10(0 min)-65/35(5.5 min)-60/40(8 min)-60/40(10 min) |
| Flow rate | : | 0.4 mL/min |
| Temperature | : | 40℃ |
| Detection | : | ESI-MS/MS(+) |
| Injection volume | : | 1 µL |
| System | : | LC: Ultimate 3000 Bio RS (Thermo Fisher Scientific K.K.); MS/MS: 3200 QTRAP (SCIEX) |
| Sample | : | 500 µg/L in $CH_3CN$ (each) |

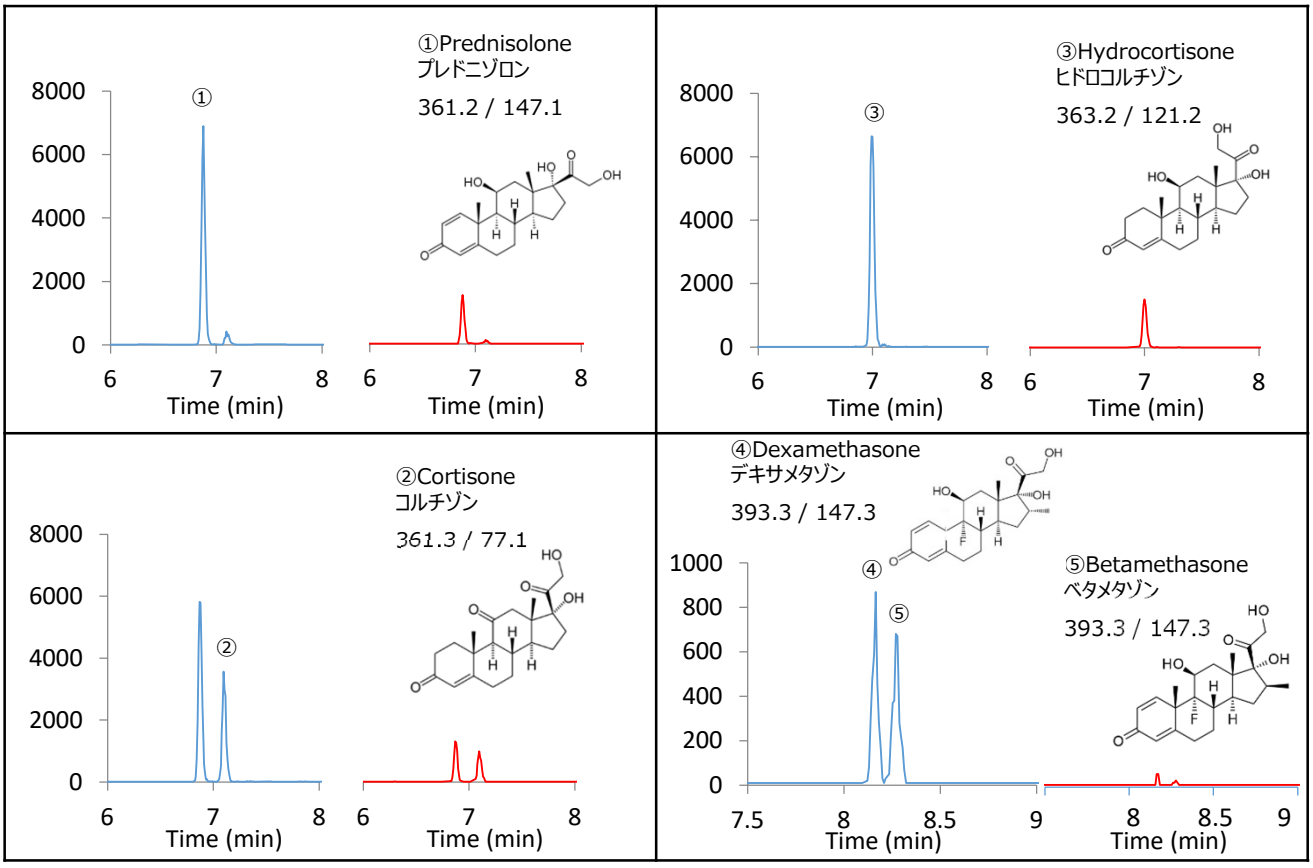


Fig. 1 Chromatogram of standard solution with different eluent
(left: 5 mmol/L $NH_3$ in $H_2O$, right: 0.1% HCOOH in $H_2O$) .

By using an ammonia eluent, the sensitivity of all steroid components was enhanced. Furthermore, the method achieved a resolution of 2.5 for betamethasone and dexamethasone, resulting in complete separation of the isomers.