High Speed Separation of Components of Steroid Drugs using an Extreme High Pressure Liquid Chromatography System (X-LC)

Y. Sato, M. Bounoshita, S. Higashidate, and M. Saito
JASCO Corporation

Betamethasone, a steroid, is administered to reduce tissue inflammation or to suppress the human immune system. The U.S. Pharmacopeia (1) (USP) method requires that HPLC analysis of components of a betamethasone drug should have a resolution, R, between the analyte and internal standard peaks to be not less than 1.7 and the relative standard deviation for replicate injections to be no greater than 2.0%.

Cortisone acetate is also a steroid that has a similar biological effect to betamethasone, and is administered to patients who have the same symptoms. Similarly, the Japanese Pharmacopoeia (2) method requires that HPLC analysis of components of a cortisone drug should have a resolution, R, between the analyte and internal standard (propyl paraben) peaks greater than 4; and the relative standard deviation for replicate injections to be no more than 1.0%.

We examined the utility of an X-PressPak C18S column (2.1 mm i.d. x 50 mm L.) packed with 2 µm diameter particle size packing material for the high speed separation of the above steroid drugs. The results were examined to determine whether the performance of the column and chromatography separation meets the USP and JP requirements.

Experimental

The chromatography system utilized in these experiments was a JASCO X-LC system consisting of a Model 3085PU HPLC pump, Model 3080DG mobile phase degasser, Model 3067CO column oven, Model 3070UV UV/Vis detector, and Model 3059AS auto sampler.

Results and Discussion

Figure 1 outlines the separation of a standard mixture of betamethazone (0.04 mg/mL) and butyl paraben (0.057 mg/mL). The X-LC system provides an analysis time 9 times shorter than conventional HPLC while the resolution between the betamethazone and butyl paraben elutions are 18.8; the reproducibility of the peak ratio is 0.44%. These results well exceed the USP requirements for the analysis.

Figure 2 illustrates the separation of a standard mixture of propyl paraben (0.03 mg/mL), butyl paraben (0.03 mg/mL), and cortisone acetate (0.1 mg/mL). In this example, the X-LC separation provides an analysis time 4 times less than conventional HPLC while the resolution between the propyl paraben and cortisone acetate elutions are 12.2; the reproducibility for the peak ratio as small as 0.44%. These results also exceed the JP requirements for the analysis.

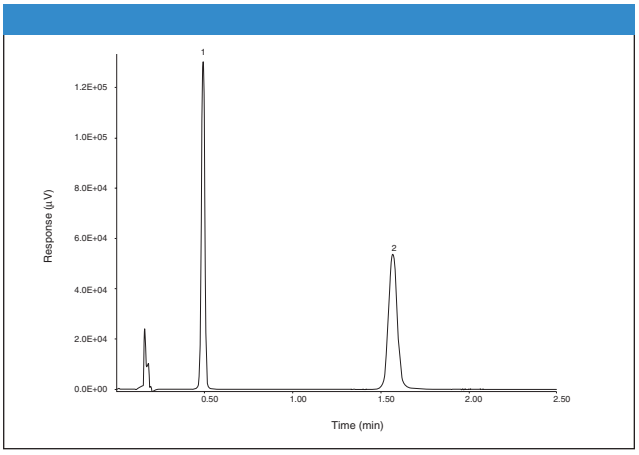

Figure 1: X-LC chromatogram of a standard mixture of betamethazone, and butyl paraben. Peaks: 1 = betamethazone (0.04 mg/mL), and 2 = butyl paraben (0.057 mg/mL). Chromatographic conditions: column = X-PressPak C18 (2.1 mm I.D. x 50 mm L); column temperature = 25 C; mobile phase = acetonitrile/water (40/60); flow rate = 0.7 mL/min; detection wavelength = 240 nm; injection volume = 1 µL.

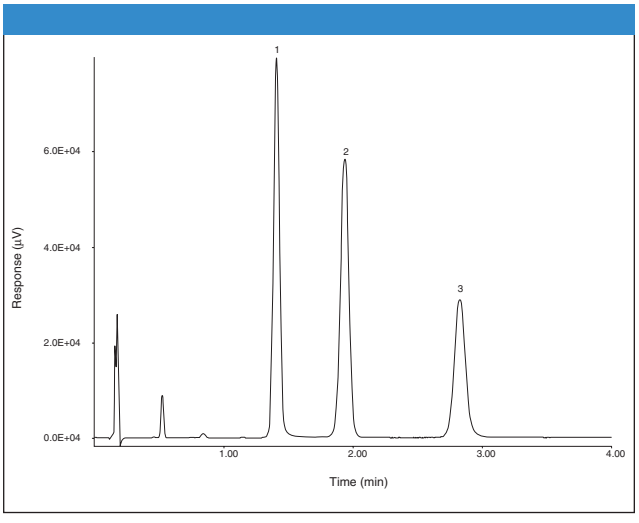

Figure 2: X-LC chromatogram of a standard mixture of propyl paraben, butyl paraben, and cortisone acetate. Peaks: 1 = propyl paraben (0.03 mg/mL), 2 = butyl paraben (0.03 mg/mL), and 3 = cortisone acetate (0.1 mg/mL). Chromatographic conditions: column = X-PressPak C18 (2.1 mm I.D. x 50 mm L); column temperature = 25 °C; mobile phase = acetonitrile/water (35/65); flow rate = 0.7 mL/min; detection wavelength = 254 nm; injection volume = 1 µL.

Jasco Inc.
8649 Commerce Drive, Easton, MD 21601
tel. (800) 333-5272
www.jascoinc.com

References

(1) *US Pharmacopeia***29**, 266 (2006)
(2) *Japanese Pharmacopeia***15**, 1423 (2006)