Application Note

747006X

High Speed Separation of Tuberculosis Medicines utilizing Extreme High Pressure Liquid Chromatography System (X-LC®)

Introduction

Tuberculosis is an infectious disease caused by mycrobacteria which attacks not only the lungs but also the central nervous system, the lymphatic system, etc.. Tuberculosis medicines include rifampin, isoniazid, and pyrazinamide.

We examined the utility of an X-PressPak C18S column (2.1 mm I.D. x 50 mm L.) packed with 2 µm diameter packing material for the ultra-high speed separation of the above drugs. The results were examined to determine whether the performance of the column and chromatography separation exceeds those of conventional HPLC.

Experimental

The X-LC system utilized in this experiment was a JASCO X-LC system consisting of two of a 3185 PU pump, 3080DG degasser, 3080MX mixing unit, 3067CO column oven, 3070UV UV/Vis detector, 3059AS auto sampler and a chromatography data system.

The conventional HPLC system was a JASCO LC-2000 Plus system consisting of a PU-2089 pump, CO-2060 column oven, UV-2070 UV/Vis detector, AS-2059 auto sampler, and a chromatography data system.

Results and Discussion

Figure 1 shows the separation of a standard mixture of rifampin (0.16mg/mL), isoniazid (0.08mg/mL), and pyrazinamide (0.43mg/mL) obtained by X-LC. Figure 2 shows the separation of the same standard mixture as in Figure 1 obtained by conventional HPLC. Table 1 illustrates results obtained from comparison of X-LC and conventional HPLC. The X-LC system provides an analysis time 6 times shorter, solvent consumtion 19 times smaller, and peak height/injection volume 11 times greater than the conventional HPLC system.

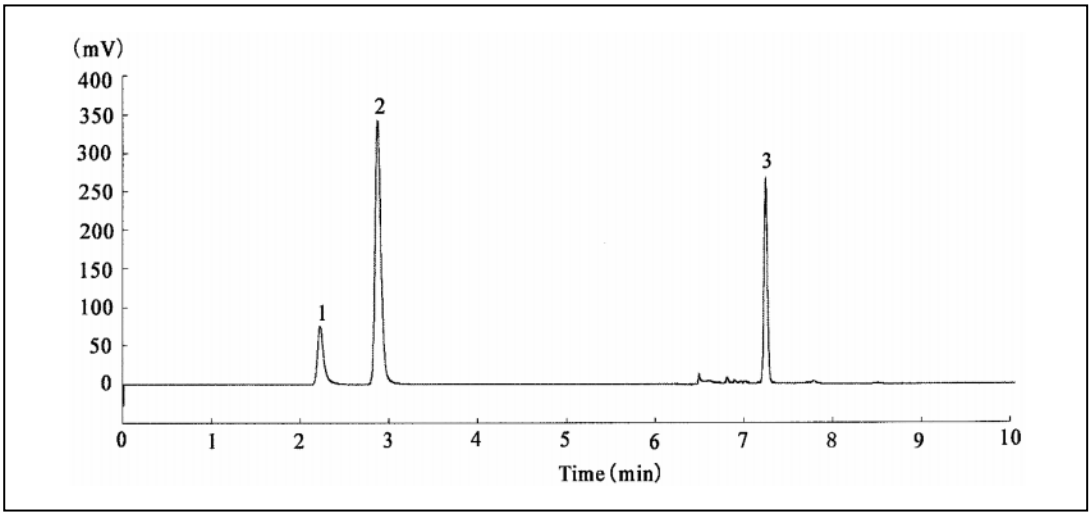

Figure 1 X-LC Chromatogram of medicines using column (4.6 mm I.D. x 150 mm) packed with 5 µm particles in diameter
Samples: 1=Rifampin(0.16 µγ/L), 2=Isoniazid(0.08 µg/L), 3=Pyrazinamide (0.43 µg/L) Injection volume: 10 µL Detection wavelength: 238 nm Flow rate: 1.5 mL/min Mobile phase: A=0.01 M Na_2HPO_4/CH_3CN(96/4, pH6.8), B=0.01 M Na_2HPO_4/CH_3CN(45/55, pH6.8) Gradient condition: 0 min, A/B(100/0); 5 min, A/B(100/0); 6 min, A/B(0/100); 15 min, A/B(0/100) Measurement pressure: 16.8-14.4 MPa

JASCO INTERNATIONAL CO., LTD
4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512

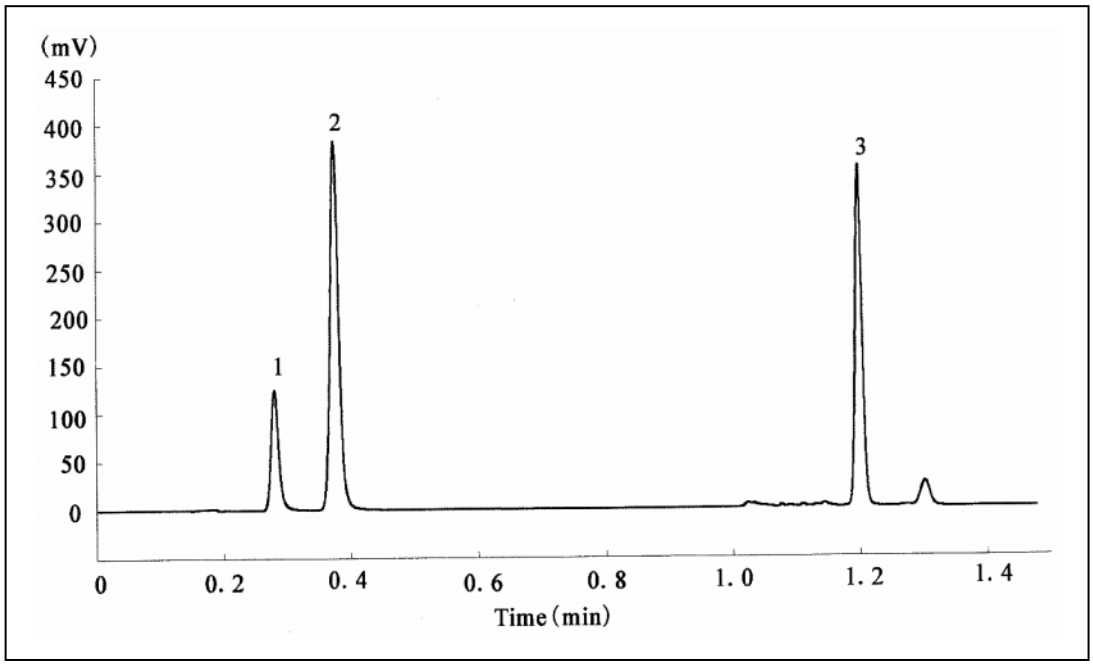

Figure 2 X-LC chromatogram of medicines using column (2.1 mm I.D. x 50 mm L.) packed with 2 µm particles in diameter
Samples: 1=Rifampin(0.16 mg/L), 2=Isoniazid(0.08 mg/L), 3=Pyraziamide (0.43 mg/L) Injection volume: 1 µL Detection wavelength: 238 nm Flow rate: 0.8 mL/min Mobile phase: A=0.01 M Na_2HPO_4/CH_3CN(96/4, pH6.8), B=0.01 M Na_2HPO_4/CH_3CN(45/55, pH6.8) Gradient condition: 0 min, A/B(100/0); 0.7 min, A/B(100/0); 0.9 min, A/B(0/100); 1.5 min, A/B(0/100) Measurement pressure: 44.9-39.1MPa

Table 1 The comparison table between conventional HPLC and X-LC

Pyrazinamide(Peak#3)	HPLC(5 µm)	X-LC (2 µm)	X-LC / HPLC
Analysis time (min.)	8	1.3	0.163
Solvent consumption (mL / 1 analysis)	30	1.6	0.053
Peak height (mAU)	337	378	1.122
Injection volume (µL)	10	1	0.1
Gradient condition (% / min.)	100	500	5

JASCO INTERNATIONAL CO., LTD
4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512