High Speed Separation of a Tuberculosis Medication Using Extreme High Pressure Liquid Chromatography (X-LC)

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High speed chromatographic separations have become increasingly important in a variety of different fields including pharmacology, food science, and agrichemistry. In general, conventional HPLC separations have been performed on columns packed with a stationary phase composed of 5 µm particles. By contrast, the JASCO X-LC Series Extreme High Pressure Liquid Chromatography system uses shorter columns packed with smaller particles on the order of 2 µm. We analyzed a tuberculosis treatment composed of three compounds (rifampin, isoniazid, and pyrazinamide) with a method outlined by USP(1) to compare the performance of X-LC with conventional HPLC.

Experimental

The conventional and high-pressure sample analyses described below were accomplished using an integrated, modular X-LC system configured for binary gradient operation with UV/Vis detection (Jasco, Hachioji, Japan).

Table I: General chromatographic analysis parameters

Mobile Phase	A: 0.01 M Na_2HPO_4/CH_3CN (96/4) (pH 6.8) B: 0.01 M Na_2HPO_4/CH_3CN (45/55) (pH 6.8)
Wavelength	238 nm
Sample	0.16 mg/L Rifampin, 0.08 mg/L Isoniazid, 0.43 mg/L Pyrazinamide (in 0.01M Na2HPO4/CH3OH (96/4))

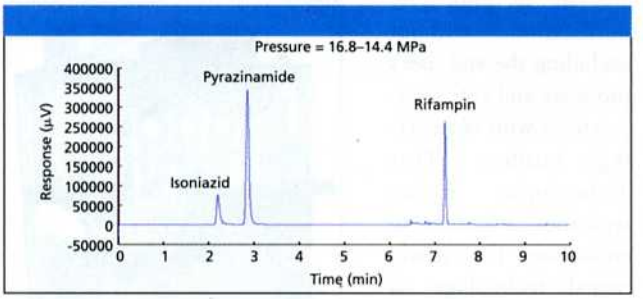

Figure 1: Chromatogram by conventional HPLC.

Figure 1 illustrates the separation of a 10 µL sample injection using conventional HPLC. A flow rate of 1.5 mL/min was used for elution of the sample using a CrestPak C18S column with a 5 µm particle size (4.6 mm i.d. × 150 mm L). The analysis time required was a little over 9 min.

Figure 2 illustrates the separation of a 1 µL sample injection using X-LC. A flow rate of 0.8 mL/min, nearly half that of the conventional HPLC separation, was used for elution of the sample using an X-PressPak C18S column with a 2 µm particle size (2.1 mm i.d. × 50 mm L). This resulted in an analysis time of 1.3 min, approximately 7 times faster than the conventional HPLC separation. The gradient elution program of 1.5 min is 10 times shorter than that of the conventional.

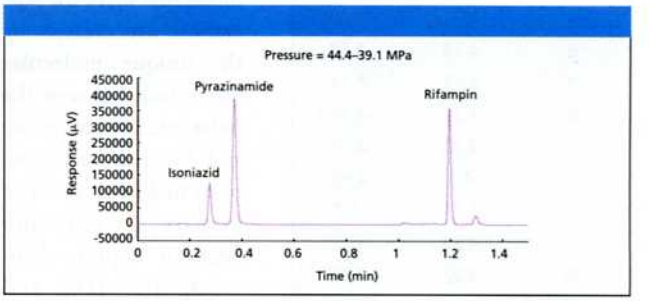

Figure 2: Chromatogram by X-LC.

Table II shows the comparison between X-LC and conventional HPLC.

Table II. Comparison between X-LC and conventional HPLC.

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

Conclusions

We have demonstrated that the X-LC system enables extremely high speed separations, up to 10 times that of conventional HPLC, with improved sensitivity and efficiency while reducing mobile phase solvent waste. This more efficient technique is also completely compatible with traditional HPLC methods providing an attractive tool for a wide range of samples.

References

(1) *U.S. Pharmacopeia* **27**, 1654 (2004).

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