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Application of Shimadzu Nexera Dual Injection (U)HPLC for the Analysis of Pharmaceuticals

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Introduction

Many people pursue ideas of “efficiency” as an ideal for daily life—ideas include how many tasks we can accomplish in a short time frame and how we can minimize our impact on the environment. The same can be true in the HPLC laboratory. The new Nexera Dual Injection (U)HPLC system equipped with two injection ports (independent flow paths) allows two analyses to be run simultaneously, and yet occupies a bench space no larger than a conventional HPLC.

To showcase the Dual Injection technique for pharmaceutical applications, the USP monographs for active pharmaceutical ingredients (API) and final pharmaceutical products were performed. Both potency and related compound analyses for active materials were completed simultaneously, while assay analysis for two different final products were also completed in parallel, which demonstrates the utility of the Dual Injection technique. The results for both analyses are stored in a single datafile which simplifies the data analysis via Multi-Data Reporting in the LabSolutions software. This work also shows the successful method transfer to superficially porous (SPP) columns for chromatographic analysis with isocratic elution. Simultaneous analyses decrease the overall analysis time by 50% while remaining compliant with USP guidelines. This work demonstrates the efficiency, throughput, and reliability of the Dual Injection system desired in high-throughput environment.

Experimental

Experiments were performed using a Nexera (U)HPLC Dual Injection system consisting of degasser DGU-405, LC-40B XR binary pump, column oven CTO-40C, photodiode array detector SPD-M40 and UV-Vis detectors SPD-40, and autosampler SIL- 40C XR configured for Dual Injection mode (Fig. 1). The data acquisition and analysis was completed with LabSolutions Chromatography Data System software version 6.99. All standards and chemicals were purchased from Sigma Aldrich and used without additional modifications.

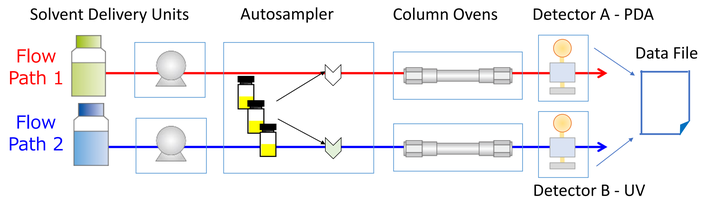


Fig. 1. Schematic diagram of Dual Injection system configuration.

USP Monograph: Clindamycin Hydrochloride Purity and Potency Analysis

Table 1. Analytical conditions for FP and SPP columns.

Column	Premier C18, 4.6x250mm, 5µm (FP)	Velox C18, 4.6x150mm, 2.7µm (SPP)
Column oven and flow cell temperature	40℃	
Detection wavelength	210 nm	
Mobile phase	0.05M Potassium Phosphate Buffer and Acetonitrile (55:45), pH 7.5	
Flow rate	1.0 mL/min	0.8 mL/min
Injection volume	5.0 µL	2.0 µL
Main peak elution time	11 min	5 min

Table 2. A) System suitability results obtained from repeated injections of standard solution of Clindamycin Hydrochloride; B) Summary for related compounds analysis for Premier (FP) and Velox (SPP) columns.

	Premier C18 (n=5)		Velox C18 (n=5)	
	Flow Stream 1	Flow Stream 2	Flow Stream 1	Flow Stream 2
R.T., % RSD	0.04	0.04	0.04	0.04
Area, % RSD	0.13	0.04	0.09	0.08
Column Efficiency	21,373	18,065	31,044	31,447
Tailing Factor	0.997	0.933	1.149	1.002
R_s (C-B vs. 7-epi)	6.5		7.7	
R_s (7-epiC vs. C)	7.1		8.5	

Related Compounds	Premier (FP), %	Velox (SPP), %
Lincomycin	0.077	0.080
Clindamycin B	0.458	0.459
7-epiclindamycin	0.224	0.228
Impurity 1	0.110	0.109
Impurity 2	0.111	0.116
Impurity 3	0.078	0.087
Total % impurities:	1.06%	1.08%

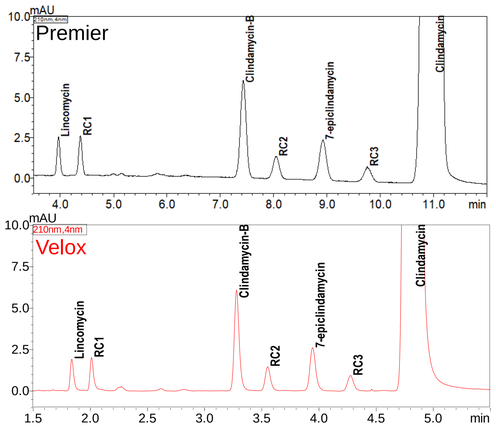


Fig. 2. Expanded chromatograms illustrating peak retention times and resolution of clindamycin hydrochloride related compounds for FP (Premier) and SPP columns (Velox).

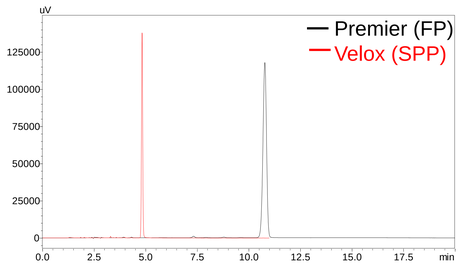


Fig. 3. Overlay of chromatograms for the potency analysis of clindamycin hydrochloride completed on FP and SPP column showing reduced run time and sharper peak with SPP column under same analysis conditions.

- ✓ Saved 50% of time with simultaneous purity and potency analysis
- ✓ Saved additional 50% of time with SPP column

USP Monograph: Guaifenesin Purity and Potency Analysis

Table 3. A) Analytical conditions for both channels; B) System suitability results.

Column	Premier C18, 4.6 x 250mm, 5 µm
Column oven and Flow cell Temp	40℃
Detection wavelength	276 nm
Mobile Phase	Water/Glacial Acetic Acid (990:10) and Acetonitrile
Gradient elution	0-32 min 20% ACN to 50% ACN
Flow rate	1.0 mL/min
Injection volume	2.0 µL

	Flow Stream 1	Flow Stream 2
R.T., % RSD	0.09	0.06
Area, % RSD	0.71	0.59
Column Efficiency	18,987	16,613
Tailing Factor	0.987	0.946
Resolution	14.8	13.8

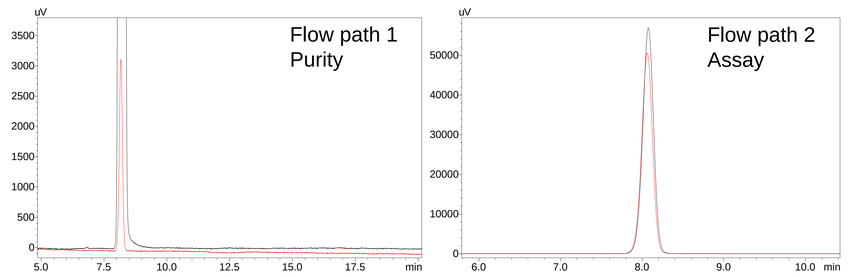


Figure 4. Expanded chromatograms for guaifenesin purity and assay analyses with flow path 1 and 2 respectively.

USP Monographs: Guaifenesin Tablets and Guaifenesin Oral Solution

Table 4. Analytical conditions for guaifenesin tablet (flow path 1, PDA) and oral solution analysis (flow path 2, UV).

	Flow Path 1, Tablet	Flow Path 2, Solution
Column	Epic C18 MS, 4.6x250mm, 10µm	Epic Polar, 4.6 x 250 mm, 10 µm
Col. oven & Flow cell Temp	40℃	
Detection wavelength	276 nm	
Mobile Phase	Water, methanol, glacial acetic acid (60:40:1.5)	
Flow rate	2.0 mL/min	2.0 mL/min
Injection volume	5.0 µL	5.0 µL
Main peak elution time	3.55 min	2.97 min

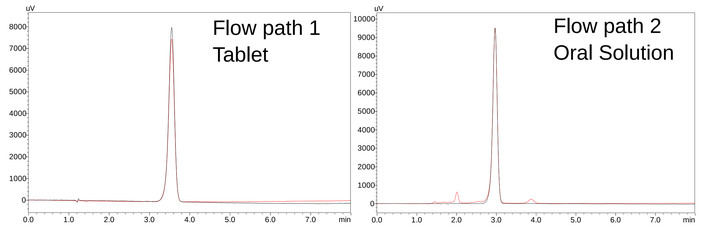
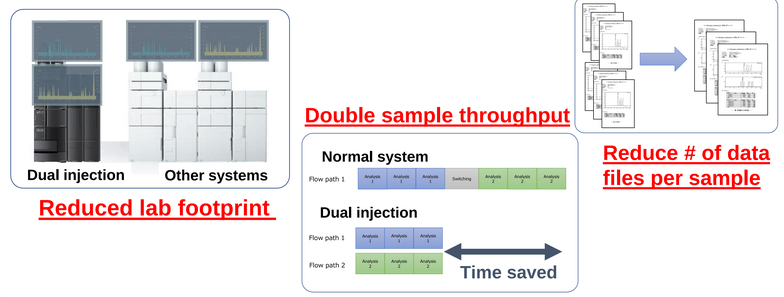


Fig. 5. Chromatograms showing assay of guaifenesin active ingredient with standard solution in black, tablet and oral solution in red. The tablet analysis was completed using flow path 1 and oral solution analysis was completed using flow path 2. The amount of API in each final product was within acceptable range of 90-110% of labeled amount.

Conclusion

This poster demonstrates that Nexera UHPLC equipped with Dual Injection System significantly improves laboratory efficiency and workflow by doubling the throughput and reducing the instrument footprint in the lab. The system allows to cut total analysis time in half by simultaneously performing the required quality control tests for finished pharmaceutical products and in-process active pharmaceutical ingredients, such as Related Compounds and Potency/Assay tests. Finally, a move to superficially porous columns for analyses using isocratic elution saved an additional 50% of the run time on the Dual Injection System, leading to further gains in efficiency.



References: USP General Chapter 621, USP 43 – NF 38, 2020