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Doubling the throughput of long chromatographic methods by using a novel Dual LC workflow

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Keywords
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Application benefits

- Dual LC technology enables the simultaneous analysis of two samples, doubling the throughput of a stability-indicating method.
- The Thermo Scientific™ Vanquish™ Flex Duo UHPLC system for Dual LC duplicates the analysis capacity per bench space in the lab.

Goal

The Vanquish Flex Duo system for Dual LC was used for the analysis of a stressed drug mixture of ezetimibe and simvastatin. It enabled the simultaneous analysis of two samples, doubling the throughput of the stability-indicating method.

Introduction

Purity analyses of drugs are routinely run in the pharmaceutical industry for purposes such as batch releases and stability studies. In most cases, reversed phase HPLC is used.

The purity analysis of drug products is frequently performed by isocratic elution. Compared to gradient methods, isocratic elution provides the required selectivity to separate related impurities with high structure similarity. Additionally, isocratic methods have better instrument portability compared to

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gradient methods. For instance, typical method transfer difficulties, such as gradient delay volume discrepancies, do not affect the transfer of isocratic methods. Still, the method must be able to retain and separate components with wide hydrophobicity range, and this results in long run times, particularly when columns packed with 5 µm particles are used. The method total run time is further increased by the column washing steps required to remove possible hydrophobic contaminants. When many samples must be processed, for instance during stability studies, long isocratic methods will decrease the number of samples that can be processed per day, extending the length of studies with obvious cost consequences and blocking of lab resources.

In this work, we introduce a novel Dual LC workflow, which provides a unique concept by using two separated flow paths in one system. The Dual LC workflow enables the simultaneous analysis of two samples by the same instrument, in practice doubling the laboratory throughput within the footprint of one instrument. The Vanquish Flex Duo system for Dual LC consists of a Dual Pump F with two individual pumping units, a Dual Split Autosampler FT with two separate injection valves and sample loops, one—or optionally two—Column Compartments H, and two detectors.

The value of the Dual workflow is here demonstrated for an isocratic stability-indicating method to profile the combined impurities of simvastatin (SMV, Figure 1) and ezetimibe (EZE, Figure 2). SMV and EZE are drugs used to reduce the total cholesterol value and triglycerides in blood. In a combinatorial therapy they are used for the

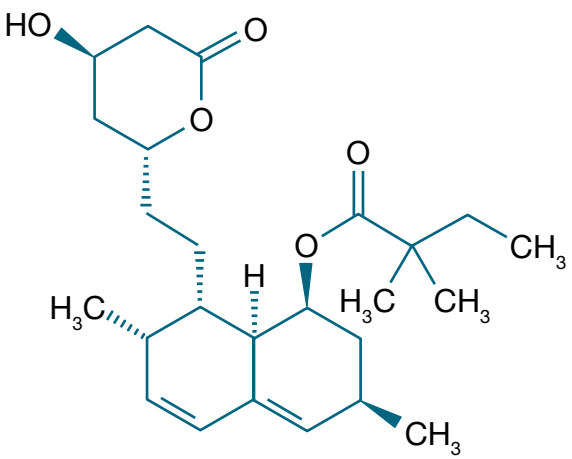

Figure 1. Chemical structure of simvastatin (SMV).

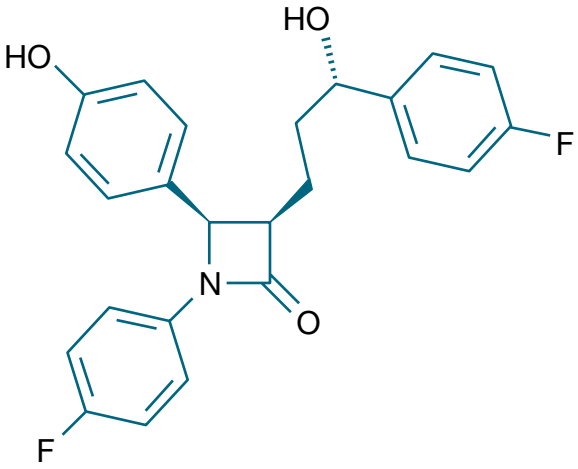

Figure 2. Chemical structure of ezetimibe (EZE).

treatment of hyperlipidemia.[1] EZE and SMV reduce the 'bad' LDL-cholesterol, while increasing the 'good' HDL-cholesterol. The LDL-cholesterol can produce serious issues to the arteria walls by building up plaques. These plaques could cause arterial occlusion, which could finally result in heart attack or stroke.

The column selected for the stability indicating method is a Thermo Scientific™ Hypersil GOLD™ PFP column. The fluorinated hydrocarbon groups provide enhanced selectivity for positional isomers of halogenated compounds, like EZE, and at the same time provide good retention for SMV and non-halogenated impurities.

Experimental

Recommended consumables

- Deionized water, 18.2 MΩ·cm resistivity
- Fisher Scientific™ Optima™ LC-MS grade acetonitrile (P/N 100001334)
- Fisher Scientific Ortho-phosphoric acid, HPLC grade (P/N 10644732)
- Fisher Scientific Sodium dihydrogen phosphate, anhydrous (P/N 12615157)
- Fisher Scientific Sodium hydroxide (P/N 10528240)
- Hypersil GOLD PFP column (250 × 4.6 mm, 5 µm) (P/N 25405-254630)
- Vials (amber, 2 mL) (P/N 15508760)
- Septa (silicone/PTFE) (P/N 11548180)

Sample handling equipment

- Fisher Scientific Ultrasonic bath
- Thermo Scientific™ Orion™ 3 Star pH meter
- Fisher Scientific Magnetic stirrer with heating option
- Fisher Scientific Conical tubes (15 mL) (P/N 11307211)
- Syringe filter, Minisart® cellulose acetate (CA) (Ø 26 mm; 0.45 µm pore size) (purchased from a reputable vendor)

Sample preparation

The respective drugs containing the active ingredients EZE and SMV were bought from a local pharmacy. One tablet of each drug was ground with a mortar and pestle. The powder was weighed out and transferred to a 15 mL conical tube. Then, 10 mL acetonitrile were added and the solution sonicated for 10 min at room temperature. One aliquot of each sample was filtered through a 0.45 µm CA membrane and used as a reference during the analysis.

To generate a wide spectrum of possible impurities of both drug substances, the extracts of EZE and SMV were combined and treated by a hydrolytic degradation described in Reference 1. The mixture was treated by adding 0.1 N NaOH and stirred for 30 min at 60 °C. Afterwards, the solution was filtered through a CA syringe filter with 0.45 µm pore size. This solution was used to detect the impurities.

Instrumentation

Vanquish Flex Duo system for Dual LC equipped with:

- System Base Vanquish Dual (P/N VF-S02-A-02)
- Dual Pump F (P/N VF-P32-A-01)
- Dual Split Sampler FT (P/N VF-A40-A-02)
- Column Compartment H (P/N VH-C10-A-02) with 2 active pre-heaters, (P/N 6732.0110)
- 2 Variable Wavelength Detectors (P/N VH-D40-A) each equipped with a 7 mm semi-micro PEEK™ flow cell, 2.5 µL (P/N 6074.0300)

Separation conditions

Column:	Hypersil GOLD PFP (250 x 4.6 mm, 5 µm) (P/N 25405-254630)
Mobile phase A:	62% 20 mM Sodium phosphate buffer, pH 3.5 / 38% acetonitrile
Mobile phase B:	Acetonitrile

Gradient (isocratic separation with column wash):

Time (min)	*% A*	*% B*
0	100	0
55	100	0
56	20	80
66	20	80
67	100	0
75	100	0

Flow rate:	0.9 mL/min
Column temperature:	40 °C
Active pre-heater temperature:	40 °C
Injection volume:	15 µL
Autosampler temperature:	4 °C
Detector wavelength:	238 nm
Data collection rate:	2 Hz
Response time:	2 s

Data processing

The Thermo Scientific™ Chromeleon™ 7.2.8 Chromatography Data System was used for data acquisition and analysis.

Results and discussion

The Vanquish Flex Duo system for Dual LC provides two separated fluidic pathways in one instrument, as can be seen in Figure 3. Each flow path consists of a sample loop and column, and each is connected to a variable wavelength detector. The same chromatographic method described above was applied to both flow paths.

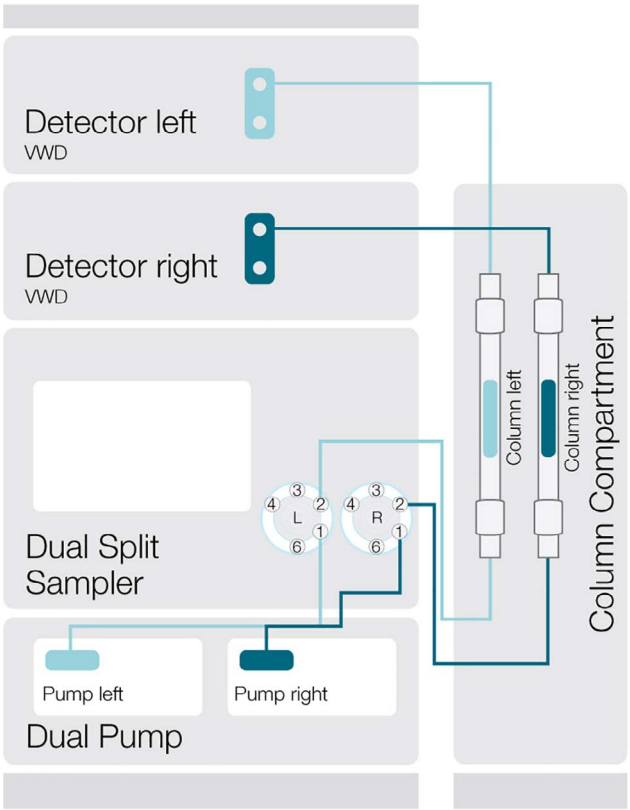

Figure 3. Schematic instrument configuration of the Vanquish Flex Duo system for Dual LC.

First, the reference samples for the EZE and SMV were run simultaneously by the Vanquish Flex Duo system for Dual LC to generate the reference chromatograms. Afterwards, the stressed sample was analyzed using the same method on both flow paths. The stress conditions generated a substantial number of impurities. In Figure 4, the chromatograms of the reference EZE sample, SMV reference sample, and the stressed EZE and SMV drug mixture are overlaid. SMV could not be detected in the stressed sample, indicating a complete degradation of this molecule under the stress conditions.

Figure 5 shows mirrored chromatograms of the stressed drug mixture analyzed with the Vanquish Flex Duo system for Dual LC using both flow paths simultaneously, whereby the same vial was used for injections into both flow paths. The chromatograms' profiles match almost perfectly.

Chromatographic consistency between the two flow paths is demonstrated by calculating the average of relative retention times of each peak for five consecutive injections. EZE is used as the reference peak for the calculation. Table 1 illustrates an almost perfect match of relative retention times obtained in the simultaneous analysis.

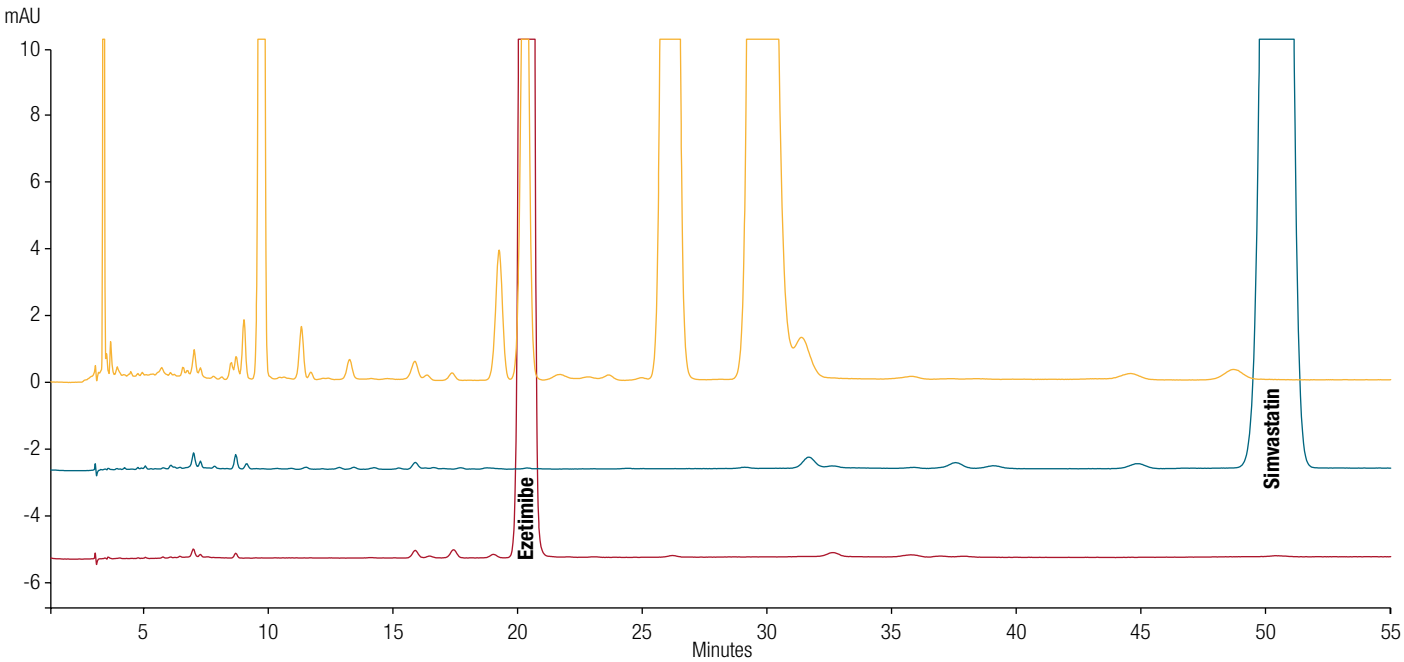

Figure 4. Chromatogram overlays of stressed mixture of EZE and SMV (orange); untreated SMV (blue), and untreated EZE (red).

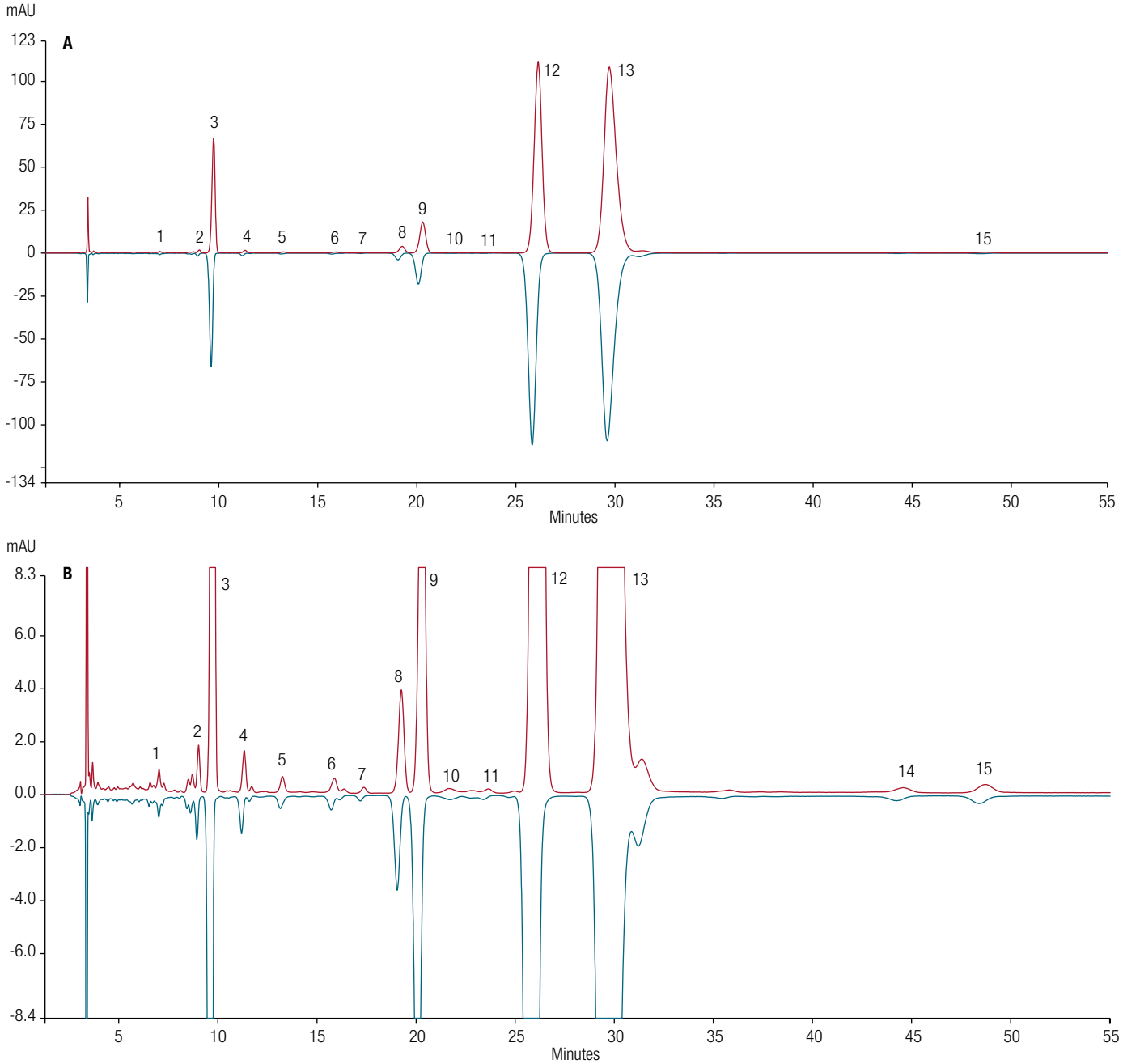

Figure 5. Mirrored chromatograms of the stressed mixture of EZE and SMV tablets (red: left flow path, blue: right flow path); Peak number assigned to all components with relative peak area > 0.05 %. A) un-zoomed view; B) zoomed view to spot related impurity peaks with lower intensity.

Table 1. Average of relative retention time with standard deviation of each peak (n=5 for each flow path). The EZE peak (peak 9) was used as the reference.

Peak	Average Relative Retention Time ± S.D. Left Flow Path	Average Relative Retention Time ± S.D. Right Flow Path
1	0.35 ± 0.001	0.35 ± 0.001
2	0.45 ± 0.002	0.45 ± 0.002
3	0.48 ± 0.002	0.48 ± 0.002
4	0.56 ± 0.002	0.56 ± 0.002
5	0.66 ± 0.002	0.66 ± 0.002
6	0.79 ± 0.003	0.78 ± 0.003
7	0.86 ± 0.005	0.85 ± 0.005
8	0.95 ± 0.005	0.95 ± 0.006
9	1.00 ± 0.006	1.00 ± 0.007
10	1.07 ± 0.009	1.07 ± 0.010
11	1.17 ± 0.008	1.16 ± 0.008
12	1.29 ± 0.008	1.28 ± 0.009
13	1.46 ± 0.012	1.47 ± 0.013
14	2.19 ± 0.015	2.20 ± 0.016
15	2.39 ± 0.020	2.40 ± 0.023

A decrease of the active ingredient EZE peak area can be observed over the five consecutive injections. On the other hand, several impurities show area increase with time (Figure 6). This indicates that the degradation process was still ongoing even when the sample was placed in the autosampler tray at 4 °C. Based on these observations, an assessment of the run-to-run peak area precision was pointless. However, the comparison of the relative peak area of two simultaneous injections is provided to evaluate the consistency of quantitative results delivered by the two channels. The data are visible in Table 2, and indicate a good agreement between the relative peak areas.

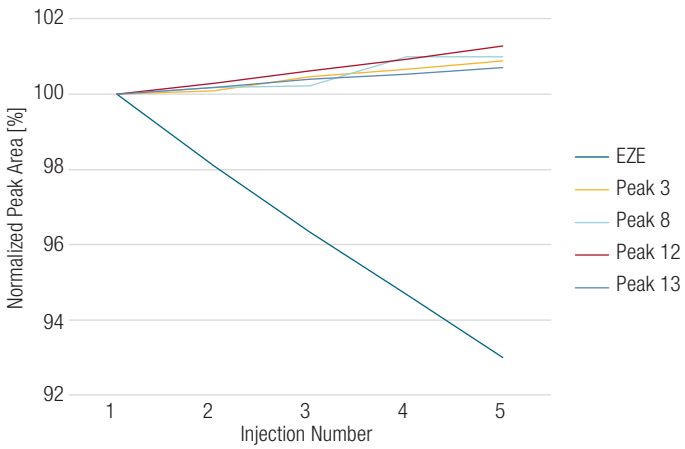

Figure 6. Normalized peak area plot over five injections, where the areas of injection 1 are normalized to 100%.

Table 2. Relative peak areas of the peaks shown in Figure 7 of the first and last injection in each flow path.

Peak	Relative Area [%] Left Flow Path	Relative Area [%] Right Flow Path	Relative Area [%] Left Flow Path	Relative Area [%] Right Flow Path
	First Injection		Last Injection	
3	6.64	6.56	6.67	6.55
8	0.67	0.67	0.67	0.67
12	27.08	26.95	27.34	26.97
13	40.67	40.67	40.83	40.45

Conclusion

- The Dual LC capabilities increased the number of analyses run on one instrument from 19 to 38 per day for this method with 75 minutes total run time.
- The Vanquish Flex Duo system for Dual LC duplicates the analysis capacity per bench space in the lab.
- Chromatographic results of both flow paths of the Vanquish Flex Duo system for Dual LC exhibit very good consistency both in relative retention time and relative peak area.

Reference

1. Dixit, R.P. et al., Stability Indicating RP-HPLC Method for Simultaneous Determination of Simvastatin and Ezetimibe from Tablet Dosage Form, *Indian J Pharm Sci,* **2010**, *72* (2), 204–210

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