

HPLC

# Simultaneous analysis of drug substances according to USP assay and impurity methods

## Authors

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## Keywords

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throughput, parallel analysis USP,  
pharmacopeial monograph, assay,  
impurities

## Application benefits

- Increased productivity on a dual-channel (U)HPLC system compared to a single-channel system
- Optimal bench space utilization with Thermo Scientific™ Vanquish™ Duo UHPLC system
- Efficient use of laboratory staff time
- Improved cost of ownership in terms of maintenance contracts after instrument warranty is over

## Goal

To demonstrate faster drug substance release by the simultaneous analysis of active pharmaceutical ingredient and its related impurities using a Vanquish Duo UHPLC system

## Introduction

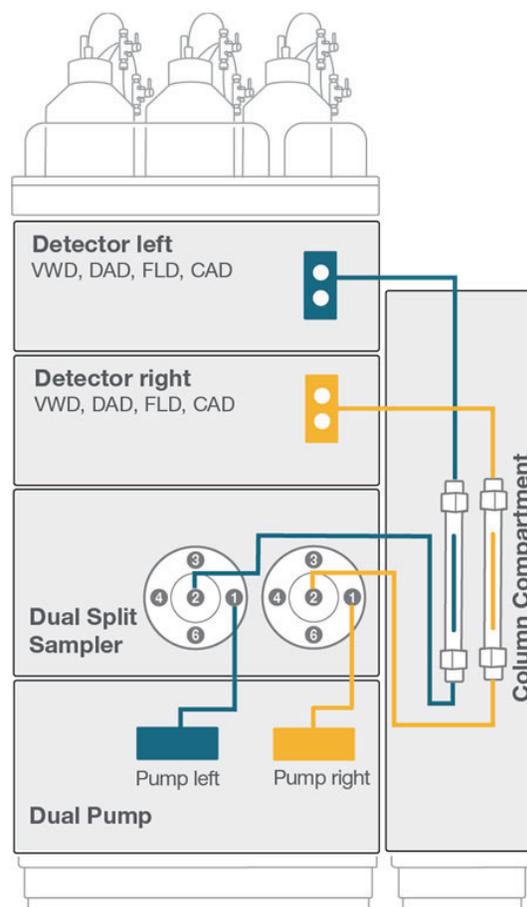
Pharmaceutical laboratories performing routine analysis of drugs are often tasked with the processing of a large number of samples. The number of HPLC systems that can run simultaneously is a key factor that dictates the turnaround time for sample processing. Therefore, labs often require numerous systems, and consequently large laboratories, to fulfill their task. The Vanquish Duo UHPLC system offers an ideal solution to significantly increase productivity.<sup>1</sup> The system provides two separate flow paths with the footprint of a single (U)HPLC system, doubling throughput while efficiently utilizing laboratory

bench space (Figure 1). Consequently, more applications can be performed with the same number of systems in one laboratory. A sample can be completely characterized for drug content and impurities with only one sample vial, one HPLC system, and one set of solvents at one time. Other aspects are a reduced number of laboratory staff and minimizing human error. For example, for eluent preparations, eluents only need to be prepared once if they are shared between both flow paths in the same system. On the other hand, a single user can only operate a limited number of instruments at the same time, even if the bench space is available. The Vanquish Duo UHPLC system therefore increases an analyst's ability to run two methods simultaneously by operating only one instrument.

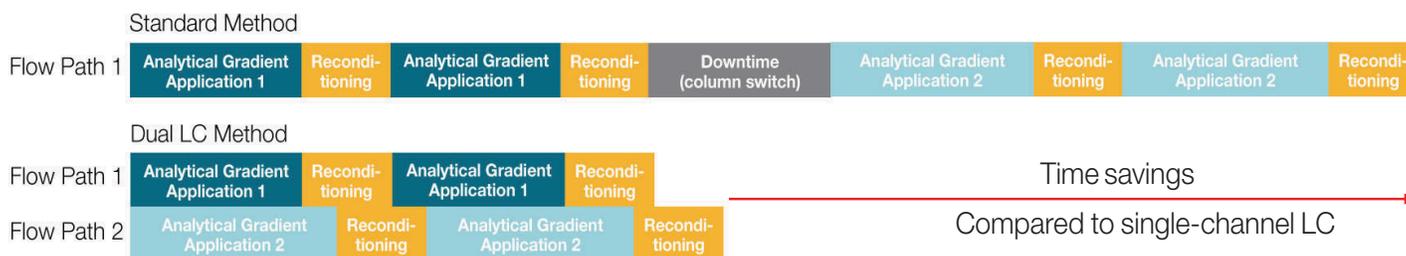
Approaches that strongly benefit from the Dual LC are:

- Two completely independent applications
- Two applications that use the same eluents but with different gradient methods and/or different target analyte concentration
- One method performed simultaneously on both flow paths to double throughput
- Method development and validation (e.g., robustness testing by testing columns from different batches)
- Mass balance study for the same set of samples of an API, where an optical (e.g., UV) and a non-optical (e.g., CAD) detector are required

This application note describes a comprehensive analysis of a drug substance using the second approach listed above. Specifically, the analysis requires two different eluent compositions for the determination of drug content and impurities of esomeprazole magnesium according to the United States Pharmacopeia (USP) monograph.<sup>2</sup> With conventional (U)HPLC systems, this would either require two different systems or sequential analyses (Figure 2).



**Figure 1. Scheme of the Vanquish Duo UHPLC system with two completely independent flow paths and two detectors; in case of the requirement of two different column temperatures, a second column compartment can be added.**



**Figure 2. Comparison between a single channel (top) and the dual channel LC (bottom) when performing two different applications**

Esomeprazole is the S-enantiomer of the racemic mixture omeprazole and belongs to a class of drugs called proton pump inhibitors (PPIs), which reduce stomach acid by the inhibition of the gastric H<sup>+</sup>/K<sup>+</sup> ATPase in parietal cells. Esomeprazole is widely used in treatments of gastro-esophageal reflux disease including erosive esophagitis, duodenal ulcer, and other gastric acid related diseases.<sup>3</sup>

## Experimental

### Chemicals

Name	Part number
Deionized water, 18.2 MΩ.cm at 25 °C, Thermo Scientific™ Barnstead™ GenPure™ xCAD Plus Ultrapure Water Purification	50136149
Acetonitrile, Optima™ LC/MS grade, Fisher Chemical™	A955
Sodium phosphate monobasic (anhydrous), for analysis, Thermo Scientific	389872500
Sodium phosphate dibasic (anhydrous), analytical reagent grade, Fisher Chemical™	S/4520/53
Sodium phosphate tribasic (anhydrous), Thermo Scientific	013438.A1
Esomeprazole magnesium (trihydrate), USP Omeprazole, USP Omeprazole Related Compound A	Purchased from a reputable vendor

### Sample handling

Name	Part number
Fisher Scientific™ Fisherbrand™ Mini Vortex Mixer	14-955-152
Vials (amber, 2 mL), Fisher Scientific	15508760
Snap Cap with Septum (Silicone/PTFE), Fisher Scientific	10547445

### Instrumentation

Module	Part number
Vanquish Flex Duo UHPLC system consisting of:	
System Base Vanquish Duo UHPLC system	VF-S02-A
Vanquish Dual Pump F	VF-P32-A
Vanquish Dual Split Sampler FT	VF-A40-A
Vanquish Column Compartment H	VH-C10-A-02
Vanquish Diode Array Detector FG (2x)	VF-D11-A
Semi-micro flow cell, 7 mm, 2.5 µL, 120 bar (2x)	6083.0530

## Preparation of standards and samples

### Assay

Sodium phosphate monobasic (0.181 g) and sodium phosphate dibasic (anhydrous) (1.118 g) were added to a 1 L volumetric flask, which was filled to volume with ultrapure water (solution A used for assay and impurities).

11 mL of 0.25 M phosphate tribasic solution and 22 mL 0.5 M sodium phosphate dibasic solution were mixed and diluted with ultrapure water to 100 mL (solution B).

To 1 mg of USP Omeprazole were added 1 mL methanol, 1 mL solution B, and 18 mL ultrapure water to obtain a final concentration of 0.05 mg/mL (standard solution assay).

To 1.1 mg of esomeprazole magnesium (trihydrate) were added 1 mL methanol, 1 mL solution B, and 18 mL ultrapure water to obtain a final concentration of 0.05 mg/mL (sample solution assay).

### Impurities

18.6 mL of solution A was mixed with 11.4 mL of acetonitrile (dilution solvent).

To 0.4 mg USP Omeprazole and 0.4 mg USP Omeprazole-related compound A were added 10 mL of dilution solvent (system suitability solution impurity).

To 1.6 mg esomeprazole magnesium (trihydrate) were added 10 mL of dilution solvent (sample solution impurity).

### Chromatography Data System

Thermo Scientific™ Chromeleon™ 7.3.1 Chromatography Data System (CDS) was used for data acquisition and processing.

**Table 1. Chromatographic conditions**

Column	Thermo Scientific™ Hypersil™ BDS C8 (150 × 4.6 mm, 5 µm) P/N 28205-154630
Mobile phase	A: 0.181 g/L sodium phosphate monobasic and 1.118 g/L sodium phosphate dibasic (anhydrous) in water (solution A) B: Acetonitrile
Isocratic condition run time (Assay)	65/35 solution A/acetonitrile (v/v) 4 min
Isocratic condition run time (Impurity)	62/38 solution A/ acetonitrile (v/v) 13.5 min
Flow rate	1.0 mL/min
Column temperature	35 °C, passive pre-heater (forced air)
Sampler temperature	20 °C
Injection volume	20 µL (Assay) 25 µL (Impurity)
UV detector parameters	Detection at 280 nm Data collection rate: 10 Hz Response time: 0.05 s

## Results and discussion

Simultaneous analysis for drug content and impurity determination of esomeprazole magnesium based on the USP monograph were performed on a Vanquish Flex Duo UHPLC system, a modern UHPLC instrument with two fully independent flow paths. This allows the two different methods to be run in parallel rather than sequentially as would be the case for a single flow path system. Furthermore, a single analyst performed the analysis on one system.

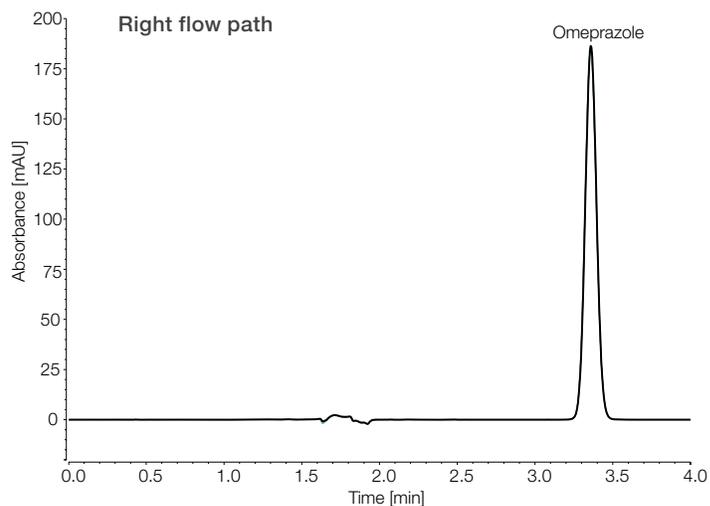
The USP monograph stipulates a L7 packing for the analyses. The Thermo Scientific Hypersil BDS C8 (150 × 4.6 mm, 5 μm) column fulfilled all required system suitability test (SST) criteria. This UHPLC instrument can easily be coupled with this conventional HPLC column, resulting in a backpressure of about 150 bar. The result showed excellent retention time and peak area reproducibility.

### Assay

The sequence began with two blank injections to determine whether there were any interferences for the target analyte region in the chromatogram. No interferences were observed. The sequence continued with six injections of the standard solution (assay) to evaluate tailing factor and relative standard deviation of peak area (RSD peak area). The sample (assay) was prepared once and injected three times. The mean of peak area response was then used for the calculation of the drug content in the sample.

Figure 3 shows six overlaid injections of the standard solution (assay). The determined tailing factor and RSD peak area were 1.04 and 0.03%, respectively, which are well below the SST requirements (tailing factor <1.5 and RSD peak area <1.0%) as outlined in the monograph. In addition, an excellent relative standard deviation of retention time (RSD RT) of 0.04% could be achieved.

Esomeprazole magnesium in the sample (assay) was calculated as 96.5% on the anhydrous basis using the formula specified in the monograph.



**Figure 3. Overlaid chromatograms of six replicate injections of the standard solution (assay) containing 0.05 mg/mL USP Omeprazole.** On a Hypersil BDS C8 (150 × 4.6 mm, 5 μm) column with phosphate buffer/acetonitrile (65/35 v/v), injection volume 20 μL, column temperature 35 °C, UV wavelength 280 nm

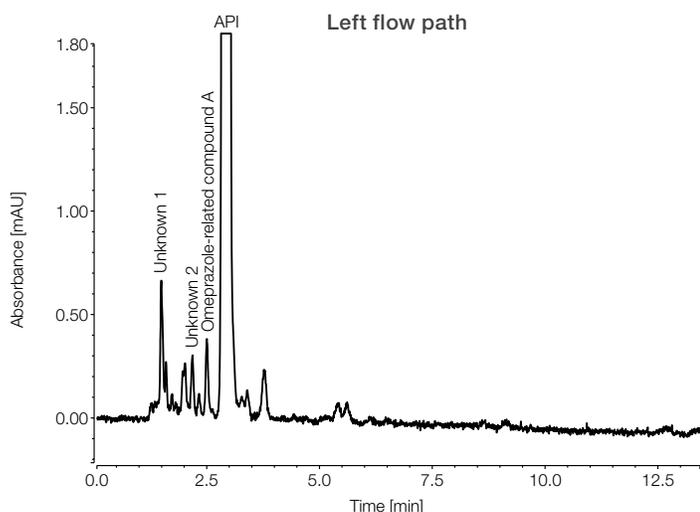
### Impurities

As for the assay method, two blank injections were performed at the beginning of the sequence and no interferences were detected. The sequence continued with six injections of the system suitability solution (impurity) to check the SST requirement for resolution between omeprazole-related compound A and omeprazole. The achieved resolution was 3.95, which easily met the requirement of more than 3.

The sample (impurity) was prepared once and injected three times. A chromatogram obtained for the sample can be seen in Figure 4. Only peaks with a signal-to-noise ratio (S/N) higher than 10 were considered for quantitative analysis. A fixed interval between 6 and 7 min was chosen for noise range determination. As shown, three impurities, one known and two unknowns, were found, with S/N for unknown 1 of 24.5, unknown 2 of 11.0, and omeprazole related compound A of 14.7.

The outlined USP requirements on relative retention time (RRT) and acceptance criteria for each impurity in comparison to the obtained results are summarized in Table 2.

As the results show, all obtained values are below the acceptance criteria and provide reliable evidence that the sample does not contain any impurities outside the limit.



**Figure 4. Chromatogram obtained for sample (impurity) containing 0.16 mg/L API.** Only peaks with signal-to-noise ratio >10 were assigned and considered for calculation; on Hypersil BDS C8 (150 × 4.6 mm, 5 μm) column with phosphate buffer/acetonitrile (62/38 v/v), injection volume 25 μL, column temperature 35 °C, UV wavelength 280 nm

## Conclusion

By performing the analyses on the Vanquish Duo UHPLC system, increased productivity was achieved, resulting in faster product release and saving bench space and laboratory staff time.

- Vanquish Duo UHPLC system enabled simultaneous execution of drug content and impurity analyses for esomeprazole magnesium in one instrument.
- Esomeprazole magnesium content in the drug sample was determined to be 96.5%.
- Impurities in the sample were determined to be below the acceptance limit of <0.5%.

## References

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2. USP monograph of esomeprazole magnesium, official as of 01-May-2021, DOI: [https://doi.org/10.31003/USPNF\\_M30588\\_03\\_01](https://doi.org/10.31003/USPNF_M30588_03_01)
3. Dong, Q.; Zhu, J.; Sui, Q.; Tang, C.; Wang, X.; Yu, Y. Optimization of mobile phase for the determination of esomeprazole and related compounds and investigation of stress condition by LC-MS, *J. Sep. Sci.* 2013, 36, 1200-1208.

**Table 2. USP requirements on impurity determination and obtained results**

Name	USP requirement		Obtained results	
	Relative Retention Time (RRT)	Acceptance criteria not more than [%]	Relative Retention Time (RRT)	Calculated value
Omeprazole N-oxide	0.45	0.1	-	-
Omeprazole-related compound A	0.8	0.2	0.85	0.04
Any other individual impurities	-	0.1	0.51	0.07
			0.74	0.03
Total impurities	-	0.5	-	0.14

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