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Application Note

A Rapid and Sensitive UPLC/UV/MS Method for Simvastatin and Lovastatin in Support of Cleaning Validation Studies

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Abstract

Utilizing ACQUITY UPLC® Technology with UV and MS detection enhances the selectivity, sensitivity, and through-put in cleaning validation studies. This results in improved methods, more confidence in results, and significant cost savings. To highlight this utility, we developed an analytical method in support of a cleaning validation study for simvastatin and lovastatin, two commonly prescribed cholesterol-lowering drugs in the statin class. Simvastatin is newly off patent and has been the focus of several generic companies. For this reason, an analytical method is needed to support cleaning validation studies in a manufacturing environment.

Introduction

Analytical Challenges

Pharmaceutical manufacturing equipment is cleaned after production to avoid cross-contamination in subsequent batches of a different product. Effectiveness of the cleaning process needs to be confirmed by

analytical measurements. Therefore, a cleaning validation method must be developed which provides evidence that cleaning processes applied to the equipment are sufficient to remove residues of bulk drug to predetermined safety levels. These methods ensure that subsequent batches of other products are not contaminated by previously manufactured products, or by the cleaning process itself. Cleaning validation methods need to achieve limits of detection which are low enough to detect small quantities of residual drug and they must demonstrate adequate resolution of drug product from interferences from solvents and the swabs or wipes used to sample the equipment surfaces. In addition, the method must be shown to be linear over the range required by the assay.

Simvastatin and lovastatin, whose structures are shown in Figure 1 are 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, which significantly impact the synthesis of cholesterol precursors. Drugs of this class represent the most efficient treatment of hypercholesterolemia. Lovastatin is a natural product, while simvastatin is semi-synthetic, being manufactured from lovastatin. As a result, lovastatin and simvastatin need to be detected in the same analytical method.

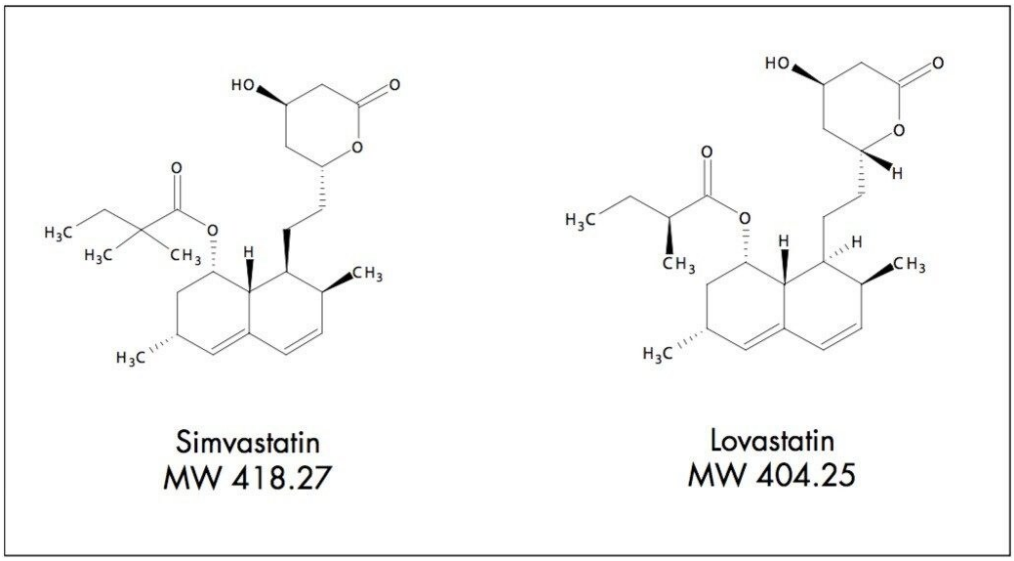

Figure 1. Structures of simvastatin and lovastatin.

Experimental

A screening approach was utilized to choose the best starting chromatographic conditions. Four different column chemistries were screened at high and low pH using two organic modifiers. The resulting chromatograms were evaluated for peak shape, retention, and selectivity. From these data, the column chemistry, pH, and mobile phase modifier were chosen. The screening gradient was later optimized to decrease run time if possible, and to improve resolution of simvastatin and lovastatin from interferences extracted from swabs or wipes.

LC Conditions for Screening

LC system:	ACQUITY UPLC with Column Manager, ACQUITY UPLC PDA and SQ
Columns:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm p/n: 186002350 ACQUITY UPLC BEH Shield RP18 2.1 x 50 mm, 1.7 µm p/n: 186002853 ACQUITY UPLC BEH Phenyl 2.1 x 50 mm, 1.7 µm p/n: 186002884 ACQUITY UPLC HSS T3 2.1 x 50 mm, 1.8 µm p/n: 186003538
Column temparature:	30 °C
Flow rate:	600 µL/min
Mobile phase A1:	0.1% HCOOH in H_2O (~pH 2.7)
Mobile phase A2:	0.1% NH_4 OH in H_2O (~pH 11)
Mobile phase B1:	MeOH
Mobile phase B2:	ACN

Gradient:	5–95% B in 2 min, hold for 0.5 min; return to initial conditions
Wavelength:	248 nm

MS Conditions

MS system:	Waters SQ Detector
Ionization mode:	ESI Positive
Capillary voltage:	3000 V
Cone voltage:	43 V
Desolvation temparature:	350 °C
Desolvation gas:	550 L/Hr
Source temparature:	120 °C
SIR channels:	Simvastatin m/z 419.25, Lovastatin m/z 405.25

Results and Discussion

Chromatographic Screening

The chromatograms resulting from pH screening with ACN as the organic modifier are shown in Figure 2. An initial PDA scan was used from 210 to 300 nm in order to determine the optimal wavelength of 248 nm for detection of simvastatin and lovastatin. All columns were run at low pH (A). The $C_{18,}$ Shield RP18, and Phenyl

columns were also run at high pH (B). (Note: the HSS T3 column is a silica particle and cannot be run at high pH.) Chromatograms were evaluated with respect to peak shape, resolution of impurities or degradants from the compounds of interest, and selectivity. Retention of these neutral compounds is relatively unaffected by pH. However, at low pH we begin to resolve additional drug compound-related peaks from simvastatin and lovastatin. The slightly broader peak shape observed on some columns at high pH is due to coelution of these smaller peaks with the compounds of interest. We chose low pH mobile phase due to better peak shape and improved resolution of impurities. We next screened all column chemistries using two organic modifiers. The chromatograms resulting from organic modifier screening are shown in Figure 3. All columns were run at low pH with MeOH (A) and ACN (B).

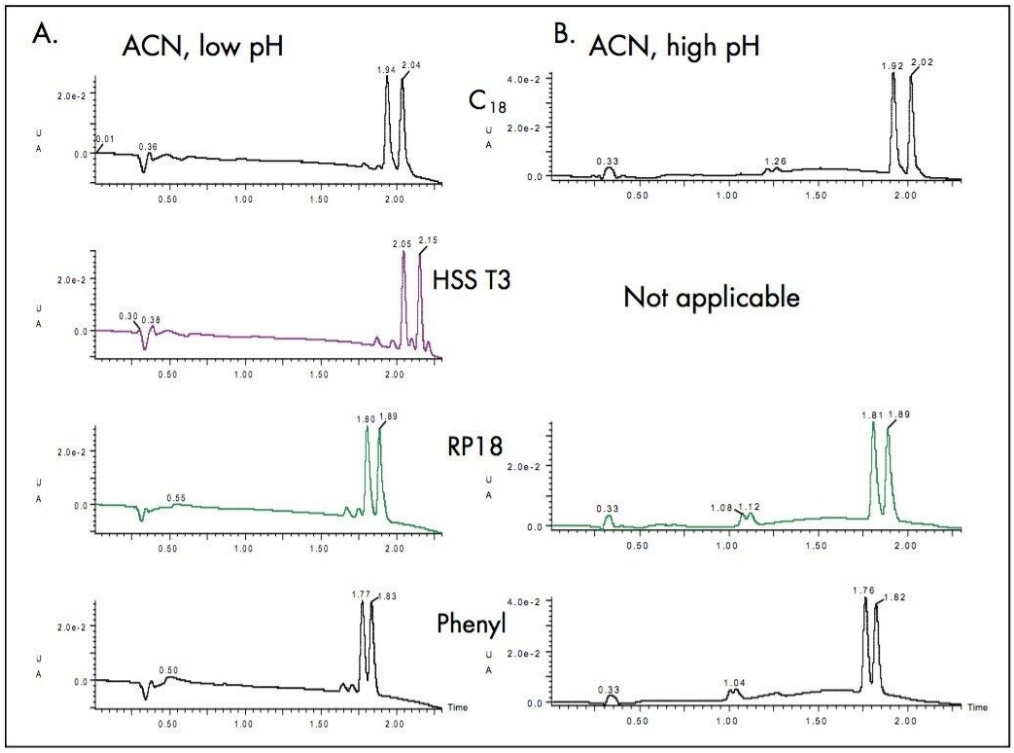

Figure 2. PDA chromatograms at 248 nm resulting from pH screening with ACN as the organic modifier.

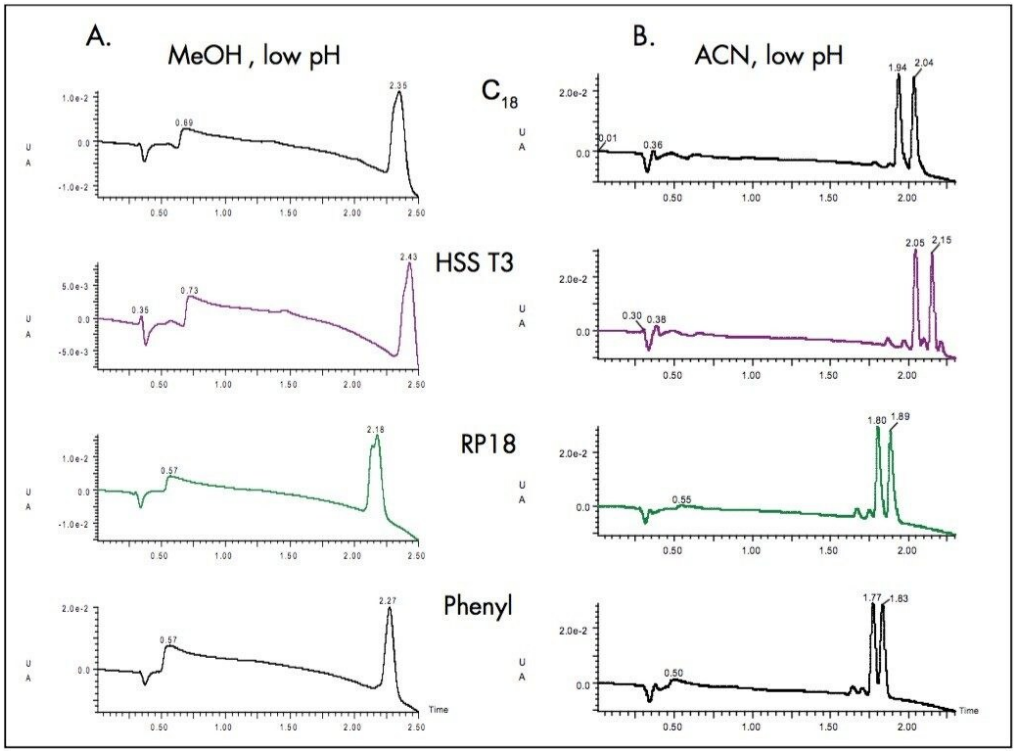

Figure 3. PDA chromatograms at 248 nm resulting from organic modifier screening at low pH.

It is clear that MeOH is not a selective enough elution solvent to resolve these hydrophobic compounds. Simvastatin and lovastatin elute as a single broad peak when MeOH is used as the organic modifier. In contrast, the two compounds chromatograph as well-resolved, narrow peaks using ACN. The final step involved choosing the optimal column chemistry. Examination of the low pH, ACN chromatograms indicates that only the HSS T3 column is able to adequately resolve all of the impurity peaks from simvastatin and lovastatin. We optimized the chromtographic method using the HSS T3 column at low pH, with ACN as the organic modifier.

Extraction Procedure

Cotton swabs (TexWipe®) were sonicated for 45 min in 10 mL of 75:25 ACN:H_2O, or 100% ACN. The extract was removed and analyzed for the presence of interferences.

Extraction Recovery

Recoveries for simvastatin and lovastatin in both the 100% ACN and 75:25 ACN:H_2O extracts were >95%. This was determined by spiking the swabs prior to extraction with known quantities of active drug. Peak area in the extract was then compared to peak area in standards prepared in the identical organic solvent.

Limit of Detection (LD) and Linear Dynamic Range

The LODs for simvastatin and lovastatin were determined under optimized chromatographic conditions with both UV and MS detection. The LOD obtained from data acquired using the PDA operating at 248 nm was shown to be 50 ng/mL for both analytes (Figure 4A). The LOD for mass spectrometry operating in SIR mode was 1 ng/mL for each analyte (Figure 4B). The dynamic range of the assay was 50 ng/mL to 50 µg/mL by PDA at 248 nm and 1 ng/mL to 1 µg/mL using mass spectrometry detection. Standard curves were linear with 1/x2 weighting over this range with R2 values from 0.983 to 0.998. All data points met the criteria for accuracy with all non-LOD points having CVs of less than ±15%, and all LOD points having CV values of less than ±20%. Sample standard curves for simvastatin and lovastatin using both PDA and mass spectrometry detection are shown in Figure 5.

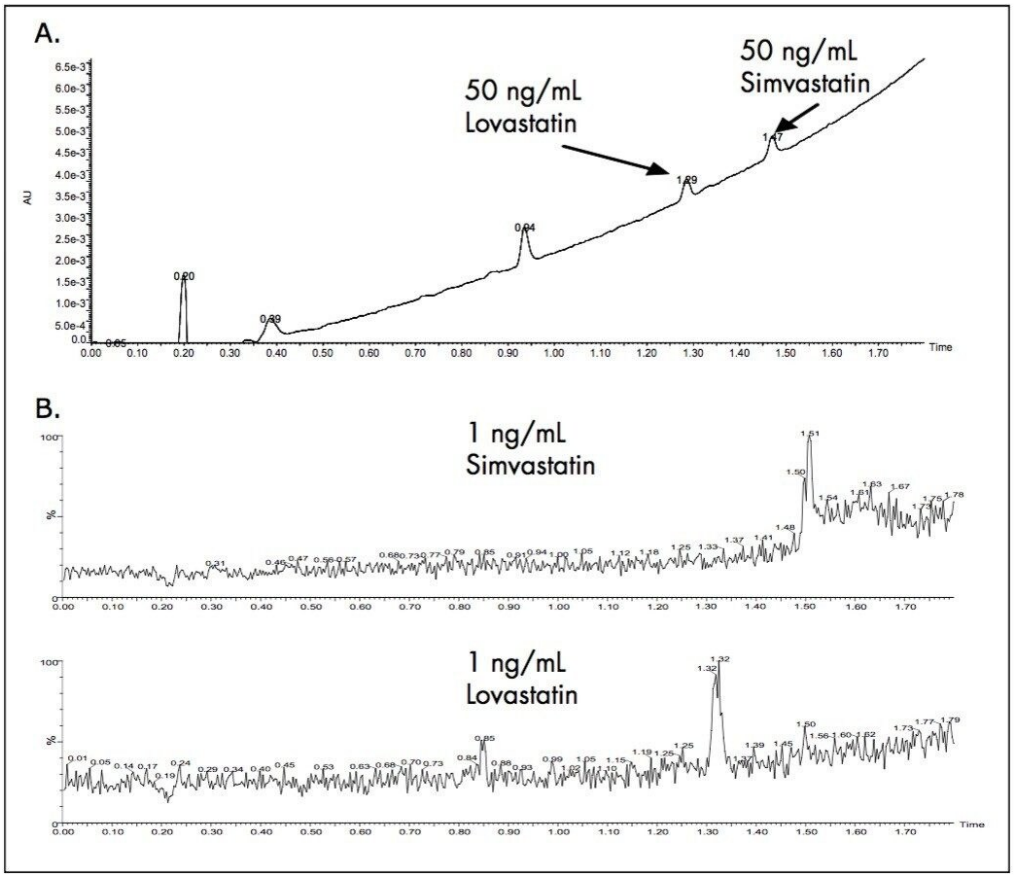

Figure 4. Limits of detection were determined for UV (A) and mass spectrometry (B.)

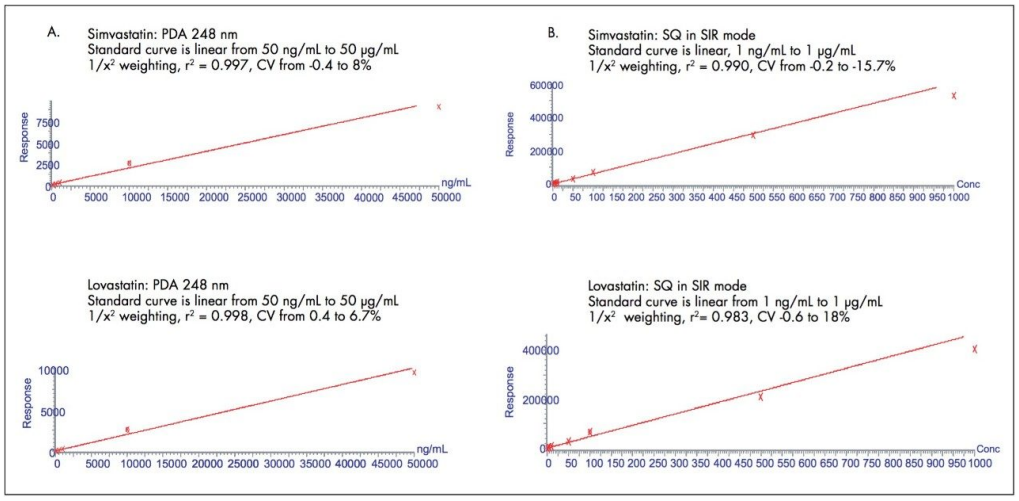

Figure 5. Standard curves for simvastatin (top) and lovastatin (bottom) using UV detection at 248 nm (A) or mass spectrometry in SIR mode (B).

Chromatographic Optimization

Simvastatin and lovastatin are very hydrophobic compounds as indicated by their late elution time under reversed-phase LC conditions. The generic screening gradient was modified to range from 50% to 95% ACN to better reflect the nature of the analytes, and to shorten the chromatographic run time. Use of this shallower gradient also allows us to improve resolution from any additional impurities or interferences discovered during analysis of swab samples. In addition, formic acid was added to the ACN (mobile phase B) at a concentration of 0.075% to reduce UV baseline drift.

Selectivity

The method was shown to be selective for the analytes of interest. Swabs were extracted as above, the extract removed, and then analyzed to confirm lack of interferences from swabs and extraction solvents. Figures 6 and 7 demonstrate that both analytes are resolved from interferences when either 100% ACN or 75:25 ACN:H_2O are used as swab extraction solvents. Figure 6 demonstrates minimal interferences using UV detection, while figure 7 demonstrates a much higher degree of selectivity for the analyte using mass spectrometry.

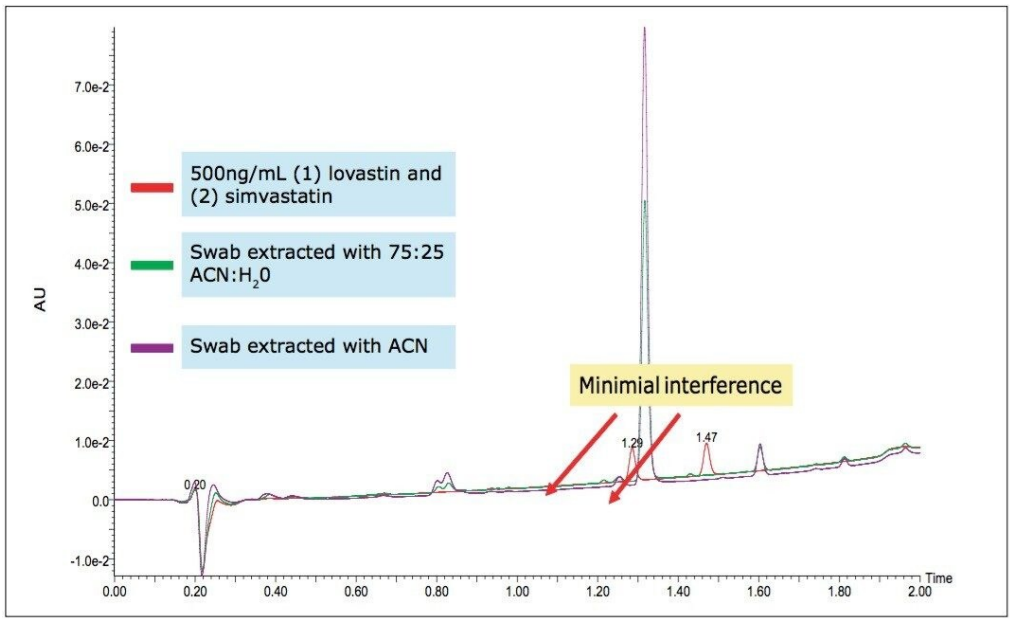

Figure 6. UV swab interferences.

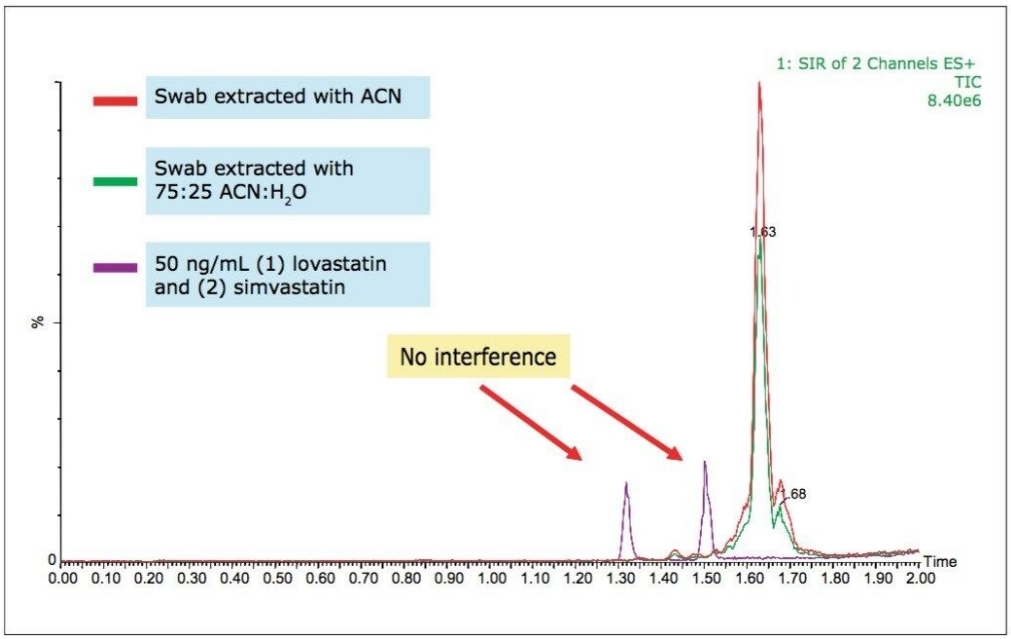

Figure 7. Mass spectrometry swab interferences.

Conclusion

We have successfully employed a chromatographic screening strategy to facilitate fast development of a rapid, selective and sensitive LC/MS/UV analytical method to support cleaning validation studies. The method achieves LODs with UV detection that meet the requirements for most cleaning validation protocols. Single quadrupole mass spectrometry yields lower limits of detection, suitable for the testing of manufacturing equipment used to prepare even very potent drugs. The increased resolution afforded by UPLC® technology allows us to separate the two analytes of interest from swab and solvent interferences in under 3 minutes. Additional selectivity is realized with mass spectrometric analysis. This rapid analysis time enables manufacturing plants to move to the next production batch faster, thus increasing their productivity and efficiency. In addition, increased simplicity and cost savings are achieved by having a single method for multiple analytes.

References

1. Additional information relating to the use of ACQUITY UPLC systems for cleaning validation studies can be found in our document entitled “ACQUITY UPLC System: Better utilizing assets in a small laboratory environment with UPLC”. Waters Application Note. 720002328.

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ACQUITY UPLC PDA Detector <https://www.waters.com/nextgen/global/products/chromatography/chromatography-detectors/acquity-uplc-pda-detector.html>

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