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Nota applicativa

Reaction Monitoring of a Rosuvastatin Synthesis Featuring Enantiopurity Determination by ACQUITY UPC^2, ACQUITY QDa, and Trefoil Column Technology

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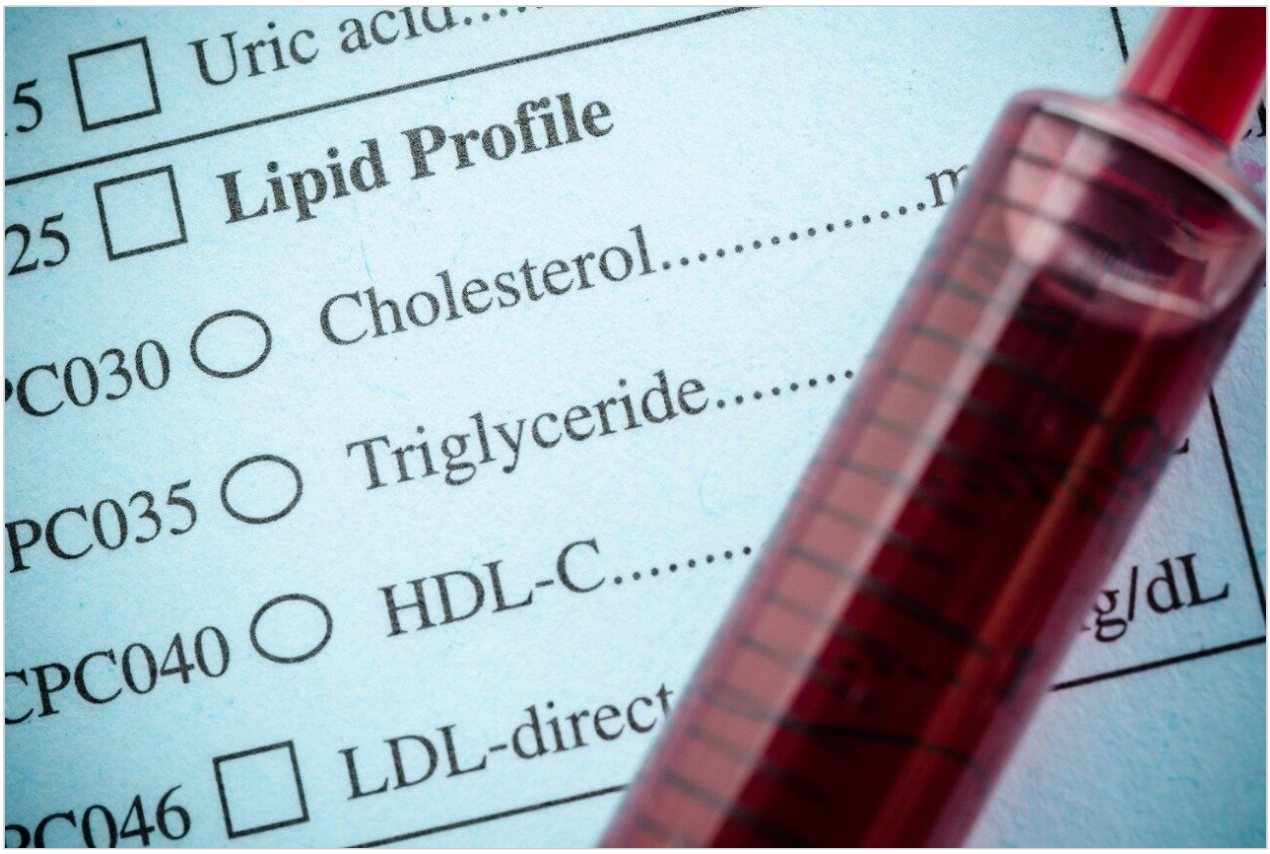

Abstract

The application note describes about reaction monitoring of the synthetic route for rosuvastatin performed utilizing an ACQUITY UPC^2 System and ACQUITY QDa Detector employed in an Open Access environment. Combining the separation power of Trefoil Columns and the ACQUITY UPC^2 System, a fast and sensitive method for determining enantiomeric excess was achieved.

Benefits

- Rapid enantiopure determinations increasing confidence in API safety and efficacy
- A single point of use capital asset solution capable of performing achiral and chiral analysis
- Robust solution for Open Access environment medicinal chemistry organizations

Introduction

Rosuvastatin (Figure 1) is an HMG-CoA reductase inhibitor marketed by AstraZeneca, aimed at therapeutically treating high cholesterol, grossing just over $5.9 billion in sales and ranking as the 10th highest revenue generating pharmaceutical drug at the end of 2014.[1] Rosuvastatin is derived from a class of six-membered heterocyclic ring compounds, specifically pyrimidine scaffolds, reported to lead to a high percentage of top marketed drugs.[2] The therapeutic form of rosuvastatin is a (3R,5S) diastereomer enantiomer and requires enantiomeric determinations. Enantiopurity, often measured as percent enantiomeric excess (%ee), reflects the relative amount of one enantiomer to another of a chiral compound. In the case for diastereomers, the purity results are typically reported as a ratio of the observed diastereomers (d.r.). For stereoselective synthesis whereas the configuration of the desired stereocenter is controlled using reagents and catalysts; with an objective to yield a single enantiomer or diastereomer, is typically reported as %ee or %de, respectively. Usually, the impurity is the less desirable enantiomer, which can alter efficacy or even toxicity. Enantiomeric determinations are a necessary analysis for all chiral drug candidates, particularly important for enantiopure formulations.

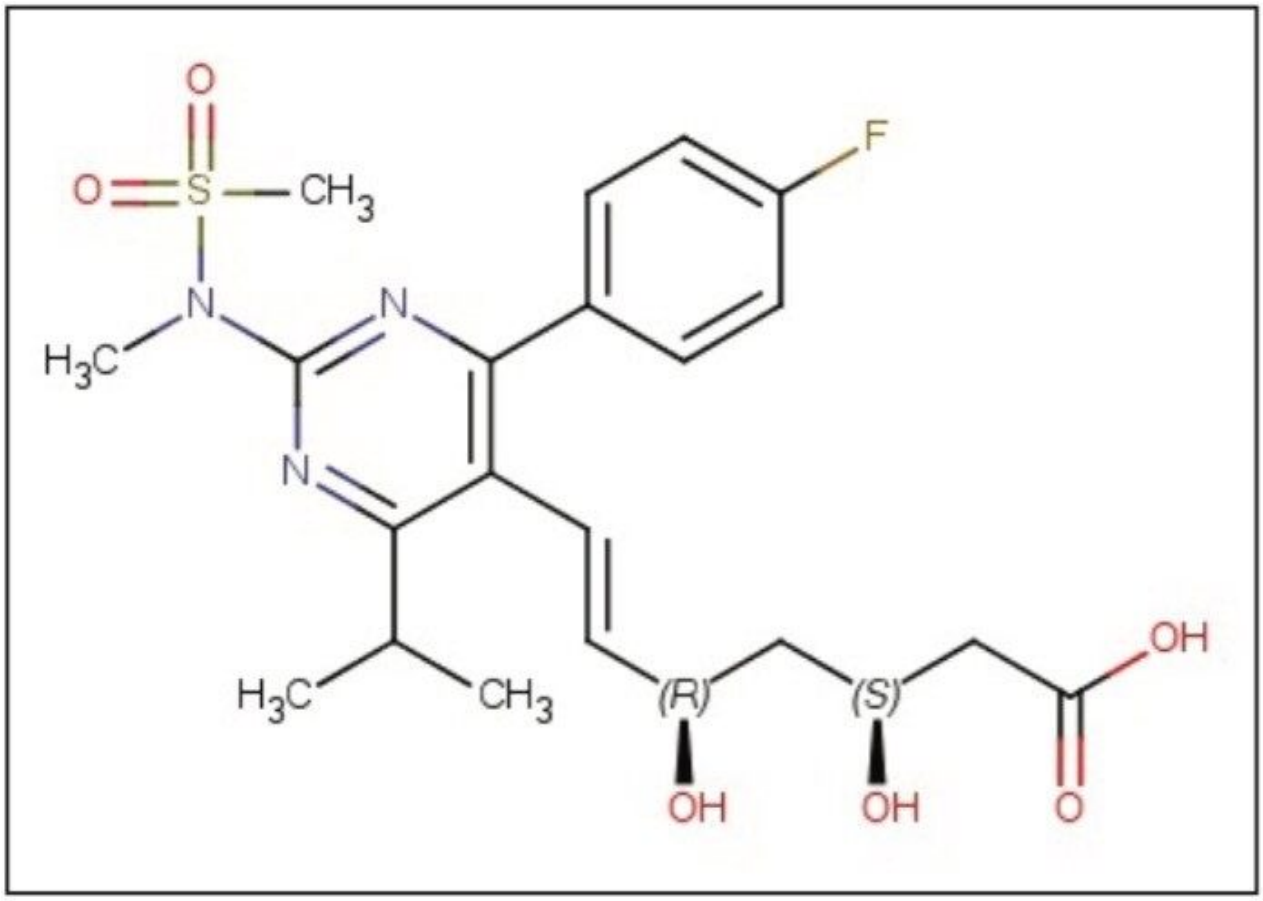

Figure 1. Structure of Rosuvastatin.

Convergence chromatography has been shown to be a particularly useful tool for achiral and chiral separations because of short analysis times and increased selectivity and resolution.[3,4] Trefoil Columns consist of efficiently packed 2.5 µm particles coated with modified polysaccharide which facilitate enantiomer separation in a chiral environment. The columns have been optimized for use on ACQUITY UPC^2 Systems and CO_2-based separations.

The rosuvastatin synthetic route includes a multi-step synthetic pathway including commonly practiced reaction mechanisms such as cyclocondensation, dehydrogenation, and modified Wittig reactions, thus leading to an enantiomeric product with the potential for a variety of impurities with each reaction intermediate. The diversity of this synthetic pathway provides a suitable example for investigating the applicability, benefits, and challenges of implementing ACQUITY UPC^2, ACQUITY QDa mass detection, and Trefoil Column technologies for chiral separations. The (d.r.) determinations specifically highlighted in this application note are focused on the final stages of the rosuvastatin reaction scheme. Monitoring the achiral and chiral compounds were performed to investigate Trefoil Columns and the ACQUITY UPC^2 System

coupled to a ACQUITY QDa Detector for mass confirmation. The aim was to assess the applicability of combining these technologies as a valuable solution for medicinal chemistry based reaction monitoring activities.

Experimental

Reaction scheme

The synthetic route developed by Hirai and Watanabe[5,6] was followed for the synthesis of rosuvastatin calcium (Figure 2). A key intermediary step of this synthetic pathway uses an aldehyde functionalized pyrimidine intermediate that allows the introduction of a modified Wittig reaction (R8) known as a Horner-Wadsworth-Emmons (HWE) reaction, to lock in the E-alkene stereochemistry of the rosuvastatin intermediate. By locking in the E-alkene stereochemistry as part of the reaction scheme, the protecting group provides access to yielding a high purity of the desirable (3R, 5S)-rosuvastatin enantiomer by desilylation of the intermediate (R9) followed by a Narasaka reduction (R10), thus producing the resulting rosuvastatin (3R, 5S) enatiomeric methyl ester. The methyl ester (R10) intermediate follows a two-step process via saponification, producing a sodium salt form of the rosuvastatin free base (R11), and then converted to the calcium salt form (R12) which is more commonly used for formulated products. Aliquots from the reaction mixtures were diluted in methylene chloride and methanol, and submitted via the OA Login wizard for each reaction monitoring time point.

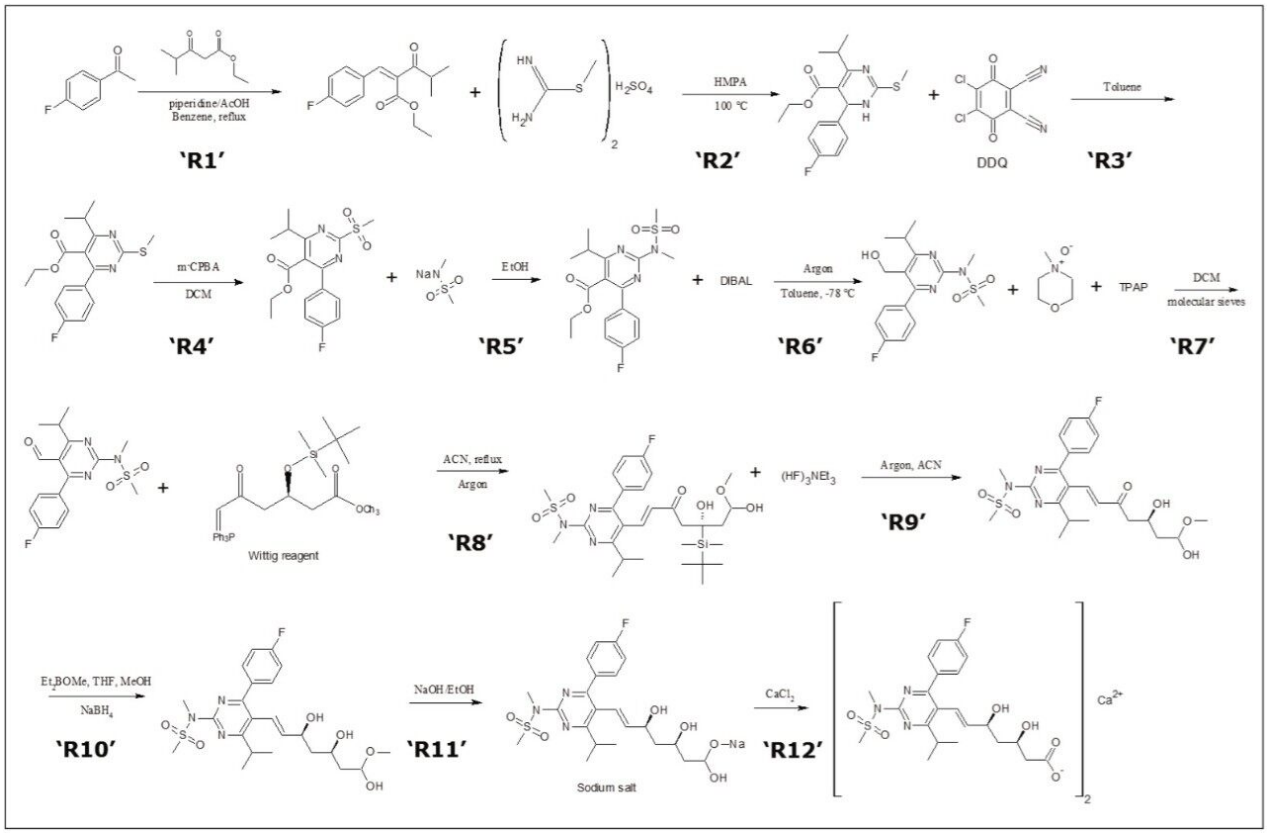

Figure 2. Reaction scheme for route synthesis of rosuvastatin calcium expanded to illustrate the reaction stages R(#) monitored by ACQUITY UPC^2 with ACQUITY QDa.

UPC2 OA screening conditions

Chromatography system:	ACQUIITY UPC^2 with ACQUITY PDA and ACQUITY QDa
Column:	ACQUITY UPC^2 BEH, 1.7 µm, 2.1 mm x 50 mm
Column temp.:	50 °C
Injection volume:	0.5 µL
Flow rate:	2.0 mL/min

UPC2 OA screening conditions

ABPR:	1885 psi
Mobile phase A:	CO_2
Mobile phase B:	MeOH w/ 15mM ammonium formate + 0.1% formic acid (v/v)
Gradient:	0–20%B over 2.6 minutes
Isocratic solvent manager (ISM):	0.3 mL/min of 0.1% ammonia in MeOH
Sampling rate:	10 Hz
Wavelength:	λ=241 nm; 350–450 λ compensated

ACQUITY QDa OA screening conditions

Ionization mode:	ESI+
Acquisition range:	MS scan 120–800 Da
Cone voltage:	10 V
Capillary voltage:	0.8 kV

UPC^2 chiral method conditions

Chromatography system:	ACQUIITY UPC^2 with ACQUITY PDA and ACQUITY QDa
Column:	ACQUITY UPC^2 Trefoil CEL1, 2.5 µm, 2.1 mm x 150 mm

UPC^2 chiral method conditions

Column temp.:	35 °C
Injection volume:	2 µL
Flow rate:	1.4 mL/min
ABPR:	1500 psi
Mobile phase A:	80% CO_2
Mobile phase B:	20% 1:1 MeOH:IPA + 20 mM ammonia
ISM:	0.3 mL/min MeOH
Sampling rate:	10Hz
Wavelength:	λ=241nm; 350–450 λ compensate

ACQUITY QDa chiral method conditions

Ionization mode:	ESI+
Acquisition range:	MS scan 250–600 Da, SIR 482.2 Da
Cone voltage:	10 V
Capillary voltage:	0.8 kV

Results and Discussion

Achiral reaction monitoring

The reaction monitoring of the earlier steps indicated the presence of a variety of related impurities, most of these were purged by chemical clean-up involving simple filtering, organic washes or recrystallization of final intermediates. In a previous application note, we observed how the selectivity of UPC^2 provided a better approach for monitoring the modified HWE reaction (R8) when compared to the UPLC reversed phase low/high pH screening results.[4] Upon review of the next reaction steps, some process impurities were observed during the desilylation (R9) of the intermediate, yet the final intermediates were calculated to be approximately 90% pure. The achiral chromatographic monitoring of the final stages (R10, R11) of the reaction scheme indicated the final products were relatively pure and free of major impurities yielding 90% and 98% purity, respectively (Figure 3). The conversion to the calcium salt (R12) is not monitored by chromatographic techniques, but rather confirmed by a simple QC check to verify completion.

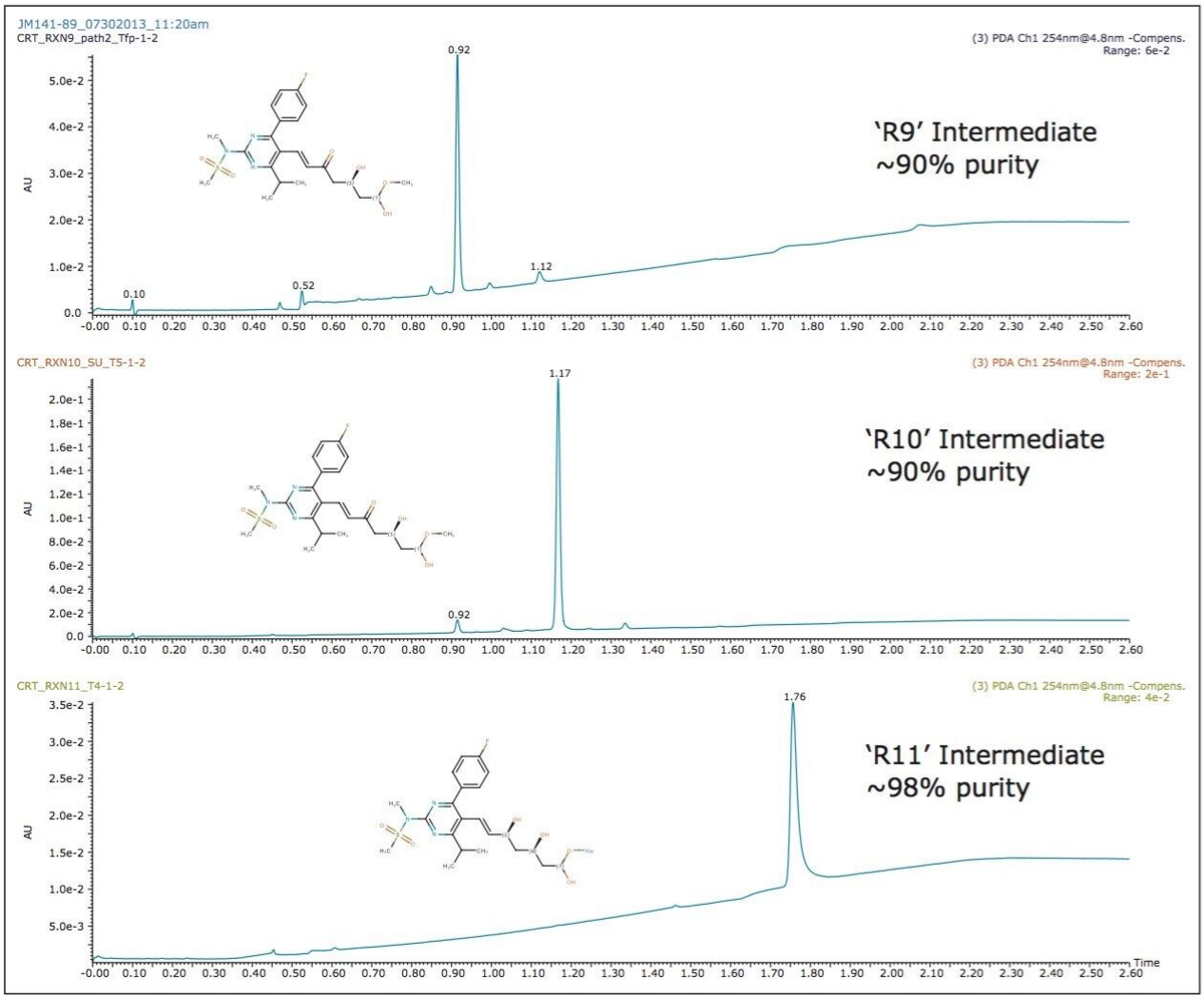

Figure 3. ACQUITY UPC^2 System with ACQUITY PDA chromatographic results overlay of intermediates R9, R10, and R11 final analysis time points

Enantiopurity determination

The enantiopurity of the rosuvastatin final product is expected to be relatively high; above 95% purity, based on the reaction scheme described in Figure 2. No major impurity peaks were observed in the chromatographic screening results of the final reactions (R11 and R12), therefore the expected risk of diastereomer impurity interferences in the final rosuvastatin product would be minimal. However, given the stereochemistry of the heptenoic side chain, a variety of diastereoisomer impurities can exist such as (3R, 5R) and (3S, 5S) including the (3S, 5R) enantiomer which requires a chiral stationary phase chromatographic separation. The original patent submission indicates a 10-minute normal phase method utilizing a Chiralpak IB Column yielding a resolution less than 2 to detect the enantiomeric impurity.[7] In addition to the poor

separation provided by the patented method, the normal phase chromatography is limited due to mass spectrometry incompatibility required for simultaneous identification and confirmation during the chromatographic separation.

A chiral chromatographic method was created to resolve rosuvastatin enantiomers using an ACQUITY UPC^2 Trefoil CEL1, 2.5 µm Column (cellulose tris-(3,5-dimethylphenylcarbamate). The final isocratic method is composed of a mixed alcohol co-solvent with a basic additive. The resolution between the enantiomers was greater than 2.0. The peaks detected by the chiral methodology were confirmed to be rosuvastatin enantiomers (m/z = 482.2 Da) as determined by the ACQUITY QDa Detector. The two diastereomer peaks were integrated and the percent diastereomer excess (%de) was calculated as:

$$\%de = \frac{E2 - E1}{E1 + E2} \text{x}100\%$$

For the 2 µL injection volumes, the d.r. was determined to be 98.7:1.3, which was above the expected 95% purity threshold. The %de was calculated to be 96.2%.

The peak areas and intensities were in good agreement with the ACQUITY QDa signal, further confirming the results obtained (Figure 4). Peak identification was confirmed by spectral analysis of the ACQUITY QDa results (Figure 5).

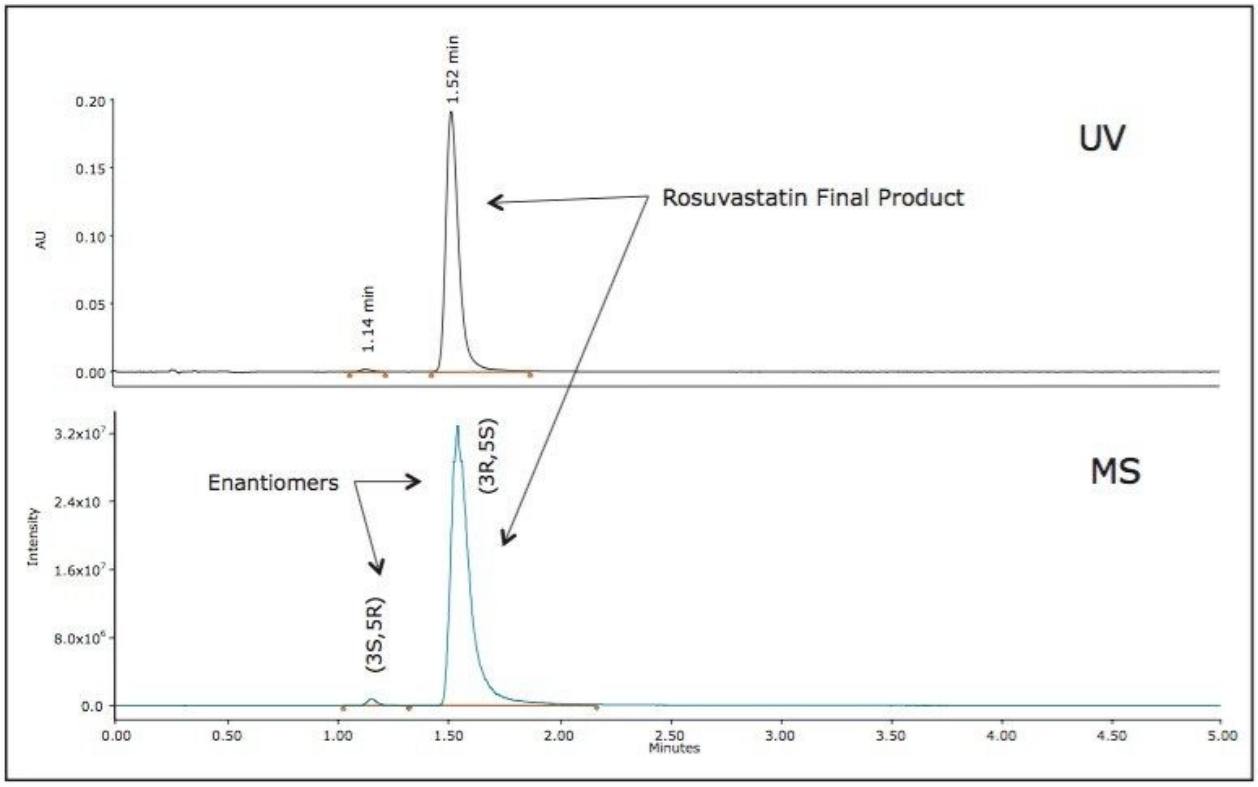

Figure 4. UV and MS chromatographic traces of the Trefoil chiral separation of 'R12' rosuvastatin reaction product.

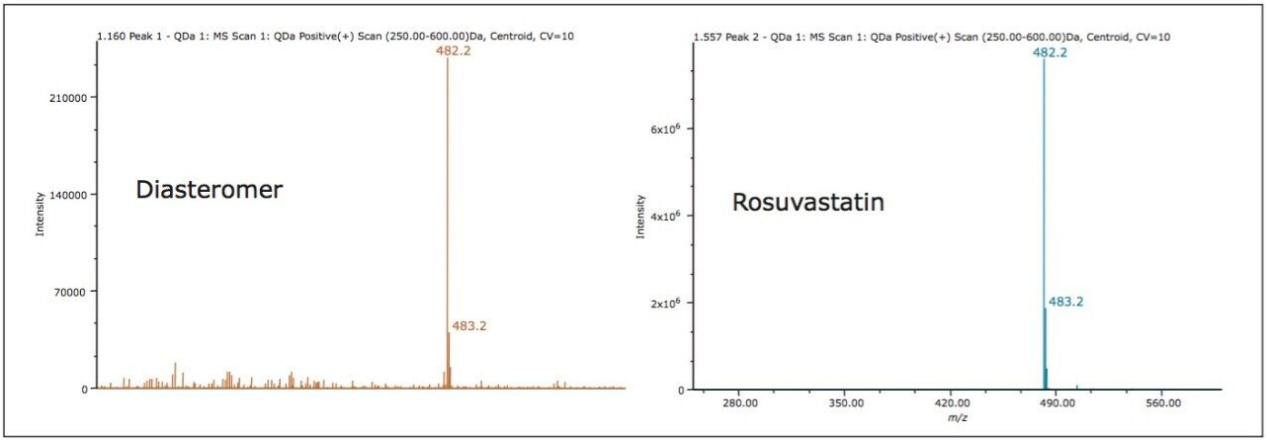

Figure 5. ACQUITY QDa MS spectra confirmed with the resulting m/z = 482 Da measurement of both peaks detected, rosuvastatin and the enantiomer.

Injection volumes from 1.0 to 9.0 µL were injected to evaluate the injection precision and observe for any

possible injection solvent effects.[8] If peak distortions are observed as a result of strong solvent diluent effects, then accurate determinations of the enantiopurity would be adversely affected and further method development would be required to mediate any peak distortion. We found the peak area to be linear (peak area R^2 = 0.9997, which is exceptional for a 10 µL loop) with minimal distortion (peak height R^2 = 0.9999) using 80/20 heptane/isopropanol as the sample diluent (Figure 6).

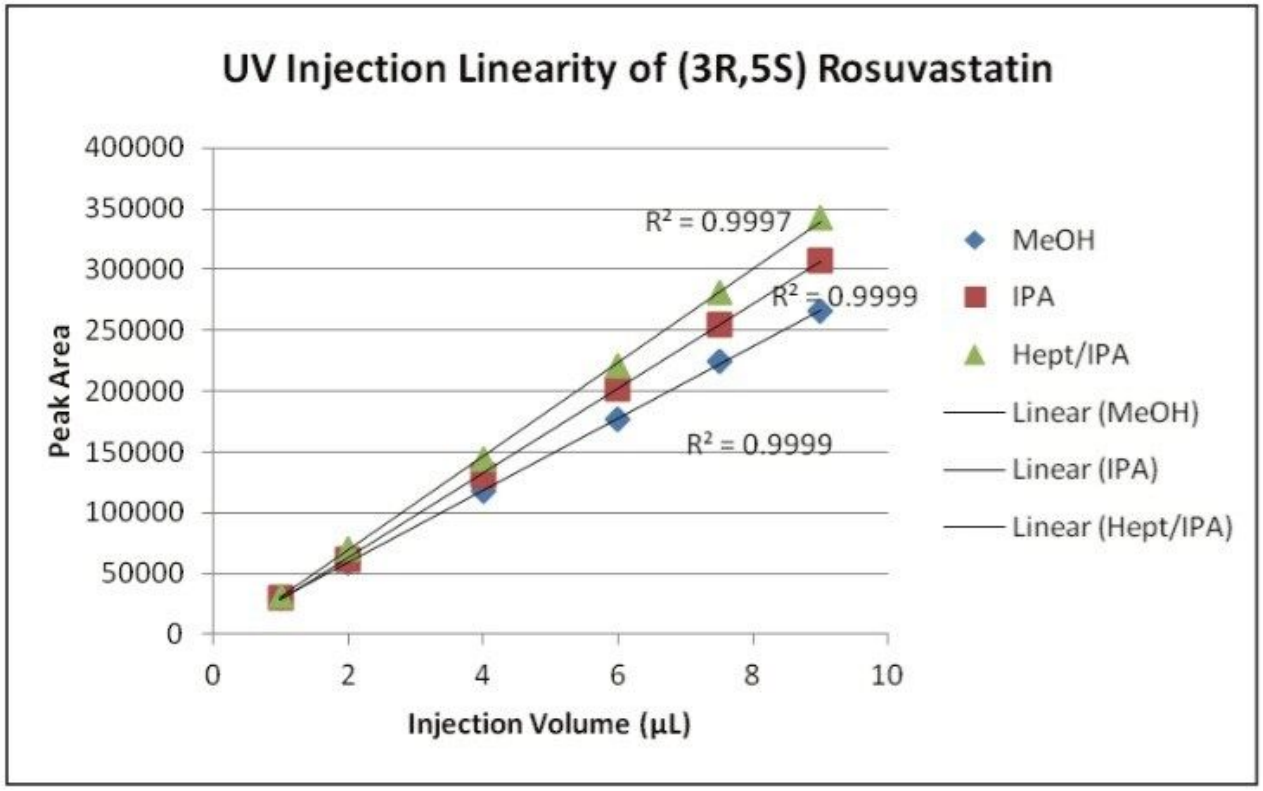

Figure 6. Injection linearity exploring injection solvent effects of rosuvastatin (3R,5S) enantiomer.

Conclusion

The achiral reaction monitoring of the synthetic route for rosuvastatin was successfully performed utilizing an ACQUITY UPC^2 System and ACQUITY QDa Detector employed in an Open Access environment. Combining the separation power of Trefoil Columns and the ACQUITY UPC^2 System, a fast and sensitive method for determining enantiomeric excess was achieved. Trefoil CEL1 delivered very good separation of the enantiomers, while providing improved resolution and MS compatibility compared to the patented

methodology. Using an ACQUITY QDa Detector for nominal mass confirmation, determining enantiomeric presence was achieved with high confidence. The Trefoil Column methodology proved to be linear at various injection volumes and free from injection solvent effects. This result provided greater confidence in the method development of the assay, which will be used for future routine use. The combined technologies of ACQUITY UPC,^{2} ACQUITY QDa, and Trefoil Columns work well to provide a fast and successful separations platform for achiral and chiral reaction monitoring.

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720005410, May 2015