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Impurity Isolation and Scale-up from UPLC Methodology: Analysis of an Unknown Degradant Found in Quetiapine Fumarate

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INTRODUCTION

A company that manufactures drug product or drug substance has a vested interest in determining the impurities associated with their compounds. The analysis of impurities can be a very labor-intensive task involving method development, impurity synthesis, isolation techniques, and various analytical approaches to determine the unambiguous identification of the impurity of interest. The lack of a pure impurity can delay a drug development program. Techniques concerning procurement of a targeted impurity are generally based on the project timelines. Impurity synthesis can be a time-consuming process requiring skilled synthetic chemists.

However, purification provides a rapid alternative to chemical synthesis that is appropriately suited to the skill set of an analytical chemist. The purification of an impurity can help with the structural elucidation by providing sufficient material for experiments such as 2D NMR. Also, collection of impurities via purification leads to reference standards of high purity.

UltraPerformance LC® provides a rapid, high-resolution approach to impurity identification and profiling. The process of scaling a UPLC® analytical method to a preparatory method can be a difficult task. Traditionally, scaling from analytical UPLC to preparatory HPLC involves calculations that transfer the flow rate and gradients associated with the original column/particle dimensions to that of the new column/particle dimensions.[1]

In this application note, a strategic approach utilizing high-resolution chromatographic theory and a forced degradation study was applied to maximize the yield of a targeted impurity of the drug substance quetiapine fumarate, an antipsychotic drug. A degradant with *m/z* 402 was found under acid stress conditions (0.1 N HCl) and chosen as the primary target for isolation. Mass-directed purification facilitated this isolation by fractionation collection of the targeted unknown impurity.

EXPERIMENTAL

In transferring the UPLC method to preparative HPLC, three key factors for the new working conditions for scale-up must be considered:

- **Separation efficiency** – L/d_p (column length/particle size) is an indication of the resolving power of the particular column. For example, a 50-mm column with 1.7-µm particles has an L/d_p of 29,411, which is equivalent to a 150-mm preparative column with 5-µm particles and an L/d_p of 30,000.
- **Productivity** – Can a shorter preparative column be utilized? In the separation of impurity *m/z* 402, a 100-mm prep column could still provide enough column efficiency to adequately isolate the impurity.
- **Column volumes** – If each of the gradient segments is scaled appropriately to maintain the equivalent number of column volumes between UPLC and preparative HPLC, the separation profile will be preserved *considering there is no change in stationary phase composition.*

Analytical conditions

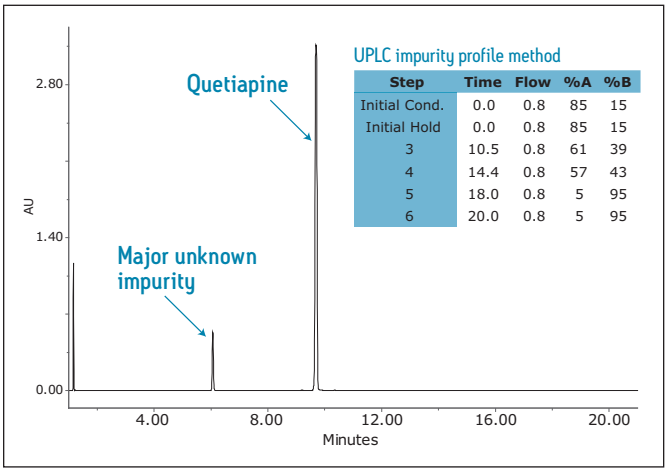
LC system:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm (optimized)
Column temp.:	Ambient
Mobile phase A:	10 mm Ammonium bicarbonate, pH 9.0
Mobile phase B:	Acetonitrile
Flow rate:	800 µL/min
Gradient (Starting):	See Figure 1
MS system:	ACQUITY® SQD
Ionization mode:	ESI positive
Capillary voltage:	1500 V
Cone voltage:	35 V
Desolvation temp.:	450 °C
Desolvation gas:	900 L/Hr
Source temp.:	150 °C
Acquisition range:	50 to 600 *m/z*

Preparative conditions

LC/MS system:	AutoPurification™ MS
Pump:	2545 Binary Gradient Module
Injector/Collector:	2767 Sample Manager
UV Detector:	2998 Photodiode Array Detector
MS Detector:	3100 Mass Detector
Column:	19 x 100 mm XBridge,™ 5 µm
Solvent A:	10 mm Ammonium bicarbonate, pH 9.0
Solvent B:	Acetonitrile
Flow rate:	25 mL/min
Gradient:	5% to 60% B over 10.5 min, flushed for approx 5 min 95% organic
Data management:	FractionLynx™ Application Manager for MassLynx™ Software

RESULTS AND DISCUSSION

A forced degradation was performed on the drug substance quetiapine. During the study, major degradants were formed for each of the various stress conditions. An impurity profile utilizing UPLC, optimized to produce maximum resolution with a 2.1 x 100 mm, 1.7 µm ACQUITY UPLC BEH C_{18} Column, was used to search for the presence of any degradants (Figure 1). Each of the major degradants was assessed by its *m/z* ratio as reported by the ACQUITY SQD single quadrupole mass detector. Peaks with masses of particular interest needed to be isolated and characterized.



Step	Time	Flow	%A	%B
Initial Cond.	0.0	0.8	85	15
Initial Hold	0.0	0.8	85	15
3	10.5	0.8	61	39
4	14.4	0.8	57	43
5	18.0	0.8	5	95
6	20.0	0.8	5	95

Figure 1. Acid hydrolysis results utilizing a highly-specific UPLC impurity profile that is optimized for maximized resolution, using a 2.1 x 100 mm, 1.7 µm ACQUITY UPLC BEH C_{18} Column.

Importance of an efficient impurity isolation process

The method was developed on an ACQUITY UPLC BEH C_{18} Column and allowed for seamless transfer to an XBridge C_{18} preparatory HPLC Column. Both XBridge HPLC particles and 1.7-µm ACQUITY UPLC BEH particles feature the same BEH Technology.

The mathematical calculations for scaling the impurity profile's UPLC methodology to preparatory conditions did not allow for an efficient isolation procedure. The L/d_p geometric scaling of the column used in the original UPLC impurity profile method resulted in a 300-mm preparatory column, as represented by the calculation:

For original UPLC quetiapine impurity profile:

$$L/d_p = 100 \text{ mm}/1.7 \text{ µm} = 58{,}823$$

Transfer to prep to maintain resolving power:

$$L/d_p = (X) \text{ mm}/5 \text{ µm} = 58, 823$$

$$X = 294 \text{ mm} \approx 300 \text{ mm column}$$

Based on this L/d_p calculation, applying the geometrically-scaled run time would result in a 60-minute preparative method – which would be excessive when considering the number of injections required to isolate milligrams of the target compound from the enriched sample preparation, thus wasting considerable time and solvent. In addition, the geometrically-scaled flow rate of 65.5 mL/min for a 19 x 300 mm column would overpressure the instrument (Figure 2). Therefore, it was necessary to make adjustments to the original UPLC impurity profile to enable a seamless scale-up.

UPLC impurity profile method

Step	Time	Flow	%A	%B
Initial Cond.	0.0	0.8	85	15
Initial Hold	0.0	0.8	85	15
3	10.5	0.8	61	39
4	14.4	0.8	57	43
5	18.0	0.8	5	95
6	20.0	0.8	5	95

Geometrically-scaled method

Step	Time	Flow	%A	%B
Initial Cond.	0.0	65.488	85	15
Initial Hold	0.3	65.488	85	15
2	31.8	65.488	61	39
3	43.5	65.488	57	43
4	54.3	65.488	5	95
5	60.3	65.488	5	95

Figure 2. Geometrically-scaled results for the preparative gradient based on quetiapine's impurity profile under UPLC gradient methodology. Calculations were performed using the Waters OBD™ Prep Calculator.

Re-optimizing for efficiency

The impurity method used to monitor the presence of degradants generated from the forced degradation study was highly specific. The forced degradation resulted in the production of only one major impurity peak with ample resolution from the active pharmaceutical ingredient (API). The decision was made to modify the impurity profile method itself to optimize for speed, while maintaining baseline resolution of the impurity peak of interest.

As stated earlier, impurity procurement is dictated by project timelines. Re-optimizing the impurity profile method to a more generic gradient satisfied analytical needs such as faster run time, lower temperature, and shorter column length (50 mm), while maintaining adequate resolution of the major impurity from quetiapine. By reducing analysis times associated with isolation, we also benefit from decreasing solvent consumption, reducing the amount of waste, and increasing sample production per unit of time.

The resulting re-optimized UPLC method utilized the same method parameters: ammonium bicarbonate at pH 9.0, ACQUITY UPLC BEH C_{18} chemistry, and acetonitrile. The peak purity of the resulting chromatogram (Figure 3) was verified by examining the mass spectral information provided by the ACQUITY SQD.

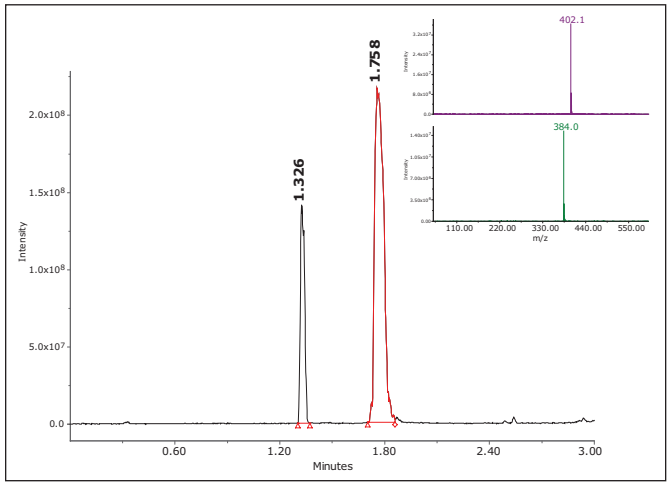


Figure 3. Re-optimized UPLC method for the peaks of interest. The resulting conditions provide an easier and more efficient solution for scaling to preparatory HPLC.

The new UPLC method with a 50-mm column provided rapid determination of the ability to use a shorter preparative column to maintain resolution. The new method resulted in an L/d_p value of 29,411, which would require a preparative column of 150 mm and a 5-µm particle size. However, after reviewing the data the observed resolution is quite large between the peaks of interest. Based on this observation, a shorter 19 x 100 mm preparative column was chosen to further decrease the preparative cycle time.

Optimizing for efficient preparative isolation

The gradient conditions for the preparative isolation analysis were optimized to maintain the same number of column volumes per gradient segment as used with the UPLC methodology. It was observed that the last peak eluted in less than two minutes, resulting in a 60% acetonitrile composition to elute all components in the re-optimized UPLC method. This observation allowed us an approach that would decrease the run time of the preparative method by applying one focused gradient segment. This practice is similar to an approach using multiple focused gradients to facilitate impurity isolation of many closely-resolved peaks.[2,3]

Step-by-step calculations were followed to determine the gradient segment durations needed for use with a 19 x 100 mm column flowing at 25 mL/min. The resulting preparative gradient is represented in Figure 4.

UPLC Column 2.1 x 50 mm, 1.7 µm	Time (min)	Flow Rate (mL/min)	%A	%B	Segment Duration Time (min)	Segment Duration (c.v)
	0.0	0.8	95	5	0	0
	2.0	0.8	40	60	2	9.25
	3.0	0.8	5	95	1	4.62

Calculated migration from UPLC to prep

Prep Column 19 x 100 mm, 5 µm	Time (min)	Flow Rate (mL/min)	%A	%B	Segment Duration Time (min)	Segment Duration (c.v)
	0.0	25	95	5	0	0
	10.5	25	40	60	10.5	9.25
	15.7	25	5	95	5.2	4.62

Figure 4. Final gradient table used to transition from UPLC to preparative analysis.

Mass-directed autopurification

The isolation of the major impurity *m/z* 402 was facilitated both analytically and chemically. In order to maximize production yield of the degradant present during the hydrolysis-forced degradation study, a stock solution of 8 g/mL quetiapine was refluxed in 0.1 N HCl for eight hours to increase the abundance of the impurity. A preparative load study allowed for 5-mL injections on-column. Together, the sample preparation and load study reduced the number of injections needed to isolate a sufficient amount of the impurity substance required for NMR analysis, while still maintaining resolution of the impurity without interferences (Figure 5).

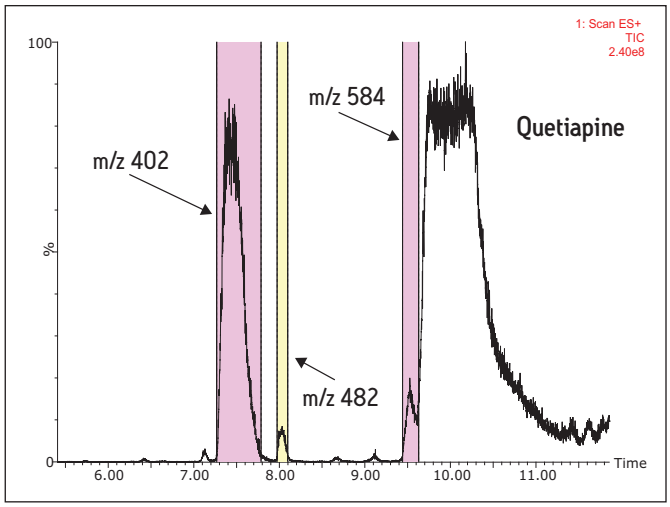


Figure 5. Preparative chromatogram of the forced degradation sample.

Using this instrumentation, automatic isolation was performed by mass triggering using the FractionLynx Application Manager for MassLynx Software. The specificity and purity of the mass triggering process was verified by UPLC/MS using the ACQUITY SQD System for the fraction analysis of the target impurity peak (Figure 6).

Re-optimizing for the impurity of interest via UPLC provided rapid methods for further analysis, such as for confirmation by UPLC/oa-Tof MS and UPLC/MS/MS.[4]

Confirmation of isolation

The isolated fractions collected for *m/z* 402 by the mass-directed purification system were pooled and evaporated to dryness. It was determined that the isolation process yielded 28.6 mg of impurity *m/z* 402. A stock solution was prepared at a concentration of 286 µg/mL, diluted to 2.86 µg/mL in methanol, and injected using the 3-minute UPLC/MS method to determine quality of the resulting isolation (Figure 6).

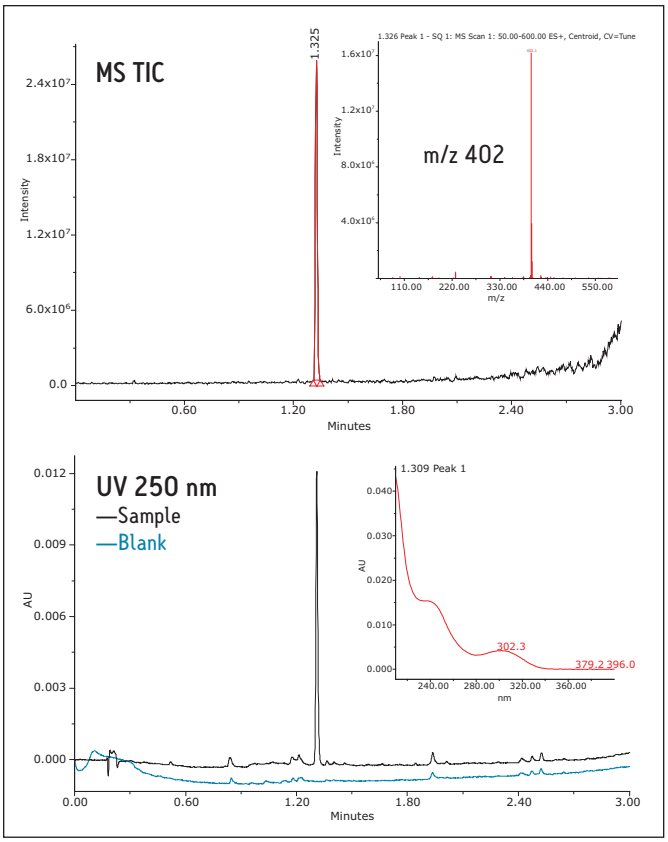


Figure 6. UPLC/MS confirmation of isolated impurity m/z 402. The resulting pooled fraction was determined to be pure, based on the MS and UV spectra that indicated zero presence of foreign substances.

CONCLUSIONS

Analytical benefits

- Maintaining L/d_p ratio was a driving factor to best manage scale-up possibilities.
- The use of a common chemistry platform for analytical-scale UPLC and preparative-scale HPLC is essential for maintaining selectivity.
- Utilizing forced degradation techniques increases production that maximizes yield.
- Mass-directed collection of the fractions assures high purity during impurity collection.
- ACQUITY SQD provides rapid confirmation of the purity composition of the pooled fractions.

Time and fiscal savings

Waters software solutions streamlined data processing and calculations:

- The dedicated FractionLynx browser presents sample and fraction information in a single, interactive location that reduced time needed to decipher data and fraction location.
- The Waters OBD Prep Calculator, a free download, facilitates scaling calculations (**www.waters.com/prepcalculator**).

Re-optimizing the UPLC method prior to preparative-scale fraction collection provided:

- A rapid and highly-specific method that utilizes less solvent and increases confidence in fraction purity.
- A scaled preparative method that requires less analysis time, considerably less solvent consumption, and less waste.
- Significant savings: 30% less time, 60% less solvent.

References

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