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Application Note

Transfer and Subsequent Redevelopment of a USP-Related Substances HPLC Method to the ACQUITY UPLC System

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Abstract

In this application note, a USP HPLC method for the analysis of mirtazapine and its related substances was first geometrically transferred to a UPLC method.

Introduction

This application note first describes a simple geometric transfer of the USP Mirtazapine Chromatographic Purity HPLC method to the Waters ACQUITY UPLC System. This system, when combined with small particle (1.7 µm) ACQUITY UPLC BEH Column chemistries, make performance gains over traditional HPLC possible. This application note then describes the redevelopment and partial validation of the previously geometrically transferred USP Mirtazapine Chromatographic Purity method and compares the two approaches. Geometric method transfer is a technique where mobile phase components, column temperature, and detection parameters

remain as originally written. Other chromatographic parameters, such as flow rate, injection volume, run time, and gradient slope (in the case of gradient separations) are mathematically scaled to the new column dimensions (length and internal diameter), as used in the ACQUITY UPLC System. This approach is simple and often provides a separation which maintains or increases resolution of critical pairs while maintaining other suitability requirements (reproducibility and tailing factors, for example). Typically, improvements in sensitivity and decreased run times are observed. Since modifications of these mathematically-scaled methods still warrant revalidation, an opportunity exists to perform a quick redevelopment and refinement of the method in order to fully exploit the benefits of the ACQUITY UPLC System and column chemistries. If a user should choose to totally redevelop the separation method, all chromatographic parameters are available for change and provides the user with an opportunity to improve legacy methods by increasing method robustness, lowering run times, fully resolving critical pairs, and lower solvent usage in a manner that was not previously available to them.

As with any newly developed method, validation experiments must performed. However, with these faster, more robust methods, combined with an easy-to-use product such as the Empower 2 Software Method Validation Manager, the entire validation process is streamlined and shortened.

Mirtazapine (1,2,3,4,10,14b-hexahydro-2-methylpyrazino[2,1-a]-pyrido[2,3-c][2]benz-azepine)(Figure 1) is an anti-depressant drug that combines the effects of increased noradrenergic activity with increased seratonergic activity by blocking α2 noradrenergic receptors and antagonizing 5-HT2 and 5-HT3 seratonergic receptors.

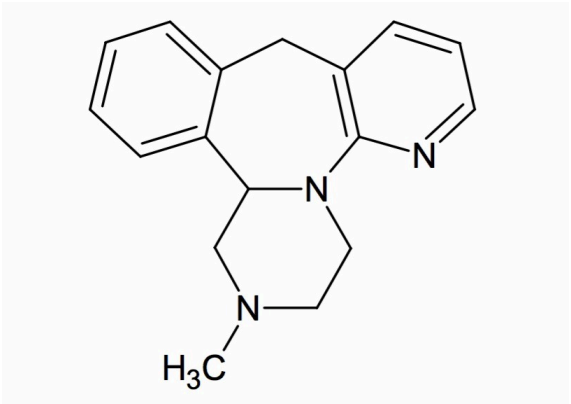


Figure 1. Mirtazapine.

Experimental

Materials

Tetramethylammonium hydroxide pentahydrate was purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile, methanol and tetrahydrofuran were purchased from Fisher Scientific (Fair Lawn, NJ). Ammonium hydrogen carbonate was purchased from Fluka (St. Louis, MO). Phosphoric acid was purchased from J.T. Baker (Phillipsburg, NJ). Water was purified with a MilliQ Gradient A10 System (Millipore, Billerica, MA). Mirtazapine and the five proprietary mirtazapine related substances were supplied by the manufacturer. Related substances were labeled simply, A through E.

HPLC Instrument and Conditions

HPLC analyses were performed on an Alliance 2695 Separations Module equipped with a 2487 Dual Wavelength Absorbance Detector. A 5 µm, 4.6 x 250 mm XBridge C_{18} Column was selected to satisfy the USP requirement for an L1 phase. The initial method conditions from the USP were a 40 °C column temperature with an injection volume of 10 µL. The isocratic separation used a tetramethylammonium hydroxide buffer, pH 7.4, mixed with Acetonitrile, Methanol, and Tetrahydrofuran in the proportion of 65:15:12.5:7.5, pumped at 1.5 mL/minute.
The detector wavelength was set to 240 nm. All samples were dissolved in a 1:1 acetonitrile/water diluent.

UPLC Instrument and Conditions

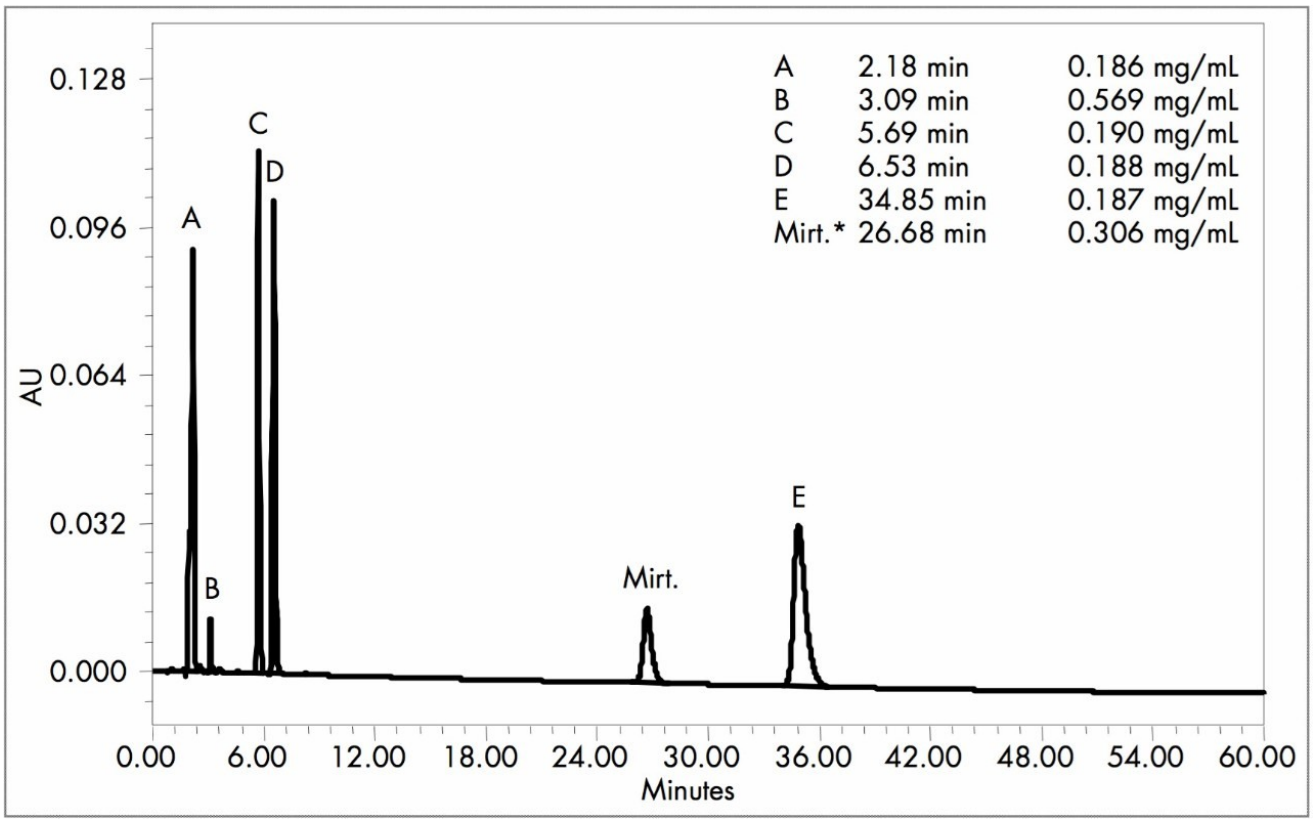
The UPLC development was performed on an ACQUITY UPLC System consisting of a Binary Solvent Manager (BSM), Sample Manager (SM) and ACQUITY UPLC Tunable UV (TUV) Detector. A 1.7 µm, 2.1 x 50 mm ACQUITY UPLC BEH C_{18} Column was selected for the separation. All Instruments described above were controlled, and data collected and analyzed using Empower 2 Software.

Results and Discussion

USP HPLC Method Verification

To verify the HPLC method, the acceptance criteria selected for this assay development from the USP were: 1) a

minimum resolution (Rs) of 2.0 for all components, 2) peak symmetry, in the form of USP tailing, less than 2.0, and 3) the %RSD of the areas from triplicate injections must not be more than 2.0%. The original method, as outlined by the USP, was evaluated and then adjusted to meet the acceptance criteria. This adjustment involved a decrease in the proportion of the organic mix in the mobile phase from 35% to 33%. A mixed standard containing mirtazapine and its five related substances, was analyzed by this method (Figure 2). All compounds were successfully resolved with a minimum Rs of 3.7 occurring between the C and D related substance peaks. Peak symmetry also met the acceptance criteria (<2.0) with USP tailing values of 1.5 and less for the related substances. The %RSD area of triplicate injections for each component was within the acceptance criterion of 2.0% (Table 1).



**Mirt. = mirtazapine*

Figure 2. Representative verified USP HPLC method chromatogram (10 µL injection).

*Parameter**	*A*	*B*	*C*	*D*	*Mirtazapine*	*E*
Mean RT min.	2.18 ± 0.18%	3.09 ± 0.23%	5.67 ± 0.50%	6.50 ± 0.46%	26.5 ± 0.54%	34.7 ± 0.40%
RRT	0.08	0.12	0.22	0.25	1.0	1.31
USP Resolution	NA	4.74 ± 1.29%	14.3 ± 1.13%	3.70 ± 1.62%	37.3 ± 0.54%	8.16 ± 0.64%
USP Tailing	0.67 ± 6.0%	1.36 ± 1.32%	1.20 ± 0.58%	1.21 ± 0.25%	1.23 ± 0.98%	1.49 ± 0.34%
%RSD Area	0.57	1.98	0.66	0.76	0.62	0.23

**RT = retention time, RRT = relative retention time*

Table 1. Results from the verified USP HPLC method [n=3].

The HPLC method was successfully verified then transferred, with appropriate scaling, to the ACQUITY UPLC System.

Transfer of the Verified USP HPLC Method to the ACQUITY UPLC System

The HPLC method was geometrically transferred to the ACQUITY UPLC System. A 2.1 x 50 mm, 1.7 µm ACQUITY UPLC BEH C_{18} Column was selected to meet the L1 USP phase requirement. The method changes required to appropriately scale the HPLC assay to the UPLC column dimensions were run time, flow rate, and injection volume. The original HPLC run time, set at approximately two times the mirtazapine retention time (as recommended by the USP), was 60 minutes.

Based on the results of these calculations, the scaled run time for the ACQUITY UPLC System was 10 minutes. The original flow rate for the USP HPLC analysis was 1.5 mL/minute. The scaled value was 0.30 mL/min. The USP HPLC injection volume of 10 µL was scaled to 0.40 µL. Following test injections, a volume of 0.10 µL was selected as a starting point as the scaled injection volume of 0.40 µL caused distortion (due to the sample diluent strength) of the very polar compound A. The equations used in the scaling process are summarized in Table 2.

Parameter	*Formula*
Run Time	$\text{UPLC Run Time} = \text{HPLC Run Time} \times \frac{\text{UPLC Column Length}}{\text{HPLC Column Length}}$
Flow Rate	$\text{UPLC Flow Rate} = \text{HPLC Flow Rate} \times \frac{(\text{UPLC Column Diameter})^2}{(\text{HPLC Column Diameter})^2}$
Injection Volume	$\text{UPLC Injection Volume} = \text{HPLC Injection Volume} \times \frac{\text{UPLC Column Volume } (\pi r^2 L)}{\text{HPLC Column Volume } (\pi r^2 L)}$

Table 2. Basic scaling equations for isocratic separations.

Transferred ACQUITY UPLC Method

The direct migration and scaling of the verified USP HPLC mirtazapine method did not effect the elution order of the components or the overall chromatographic profile (Figure 3). Resolution, tailing factor and reproducibility, in the form of %RSD of the area of triplicate injections, were all within allowable limits as defined in the original USP HPLC method (Table 3).

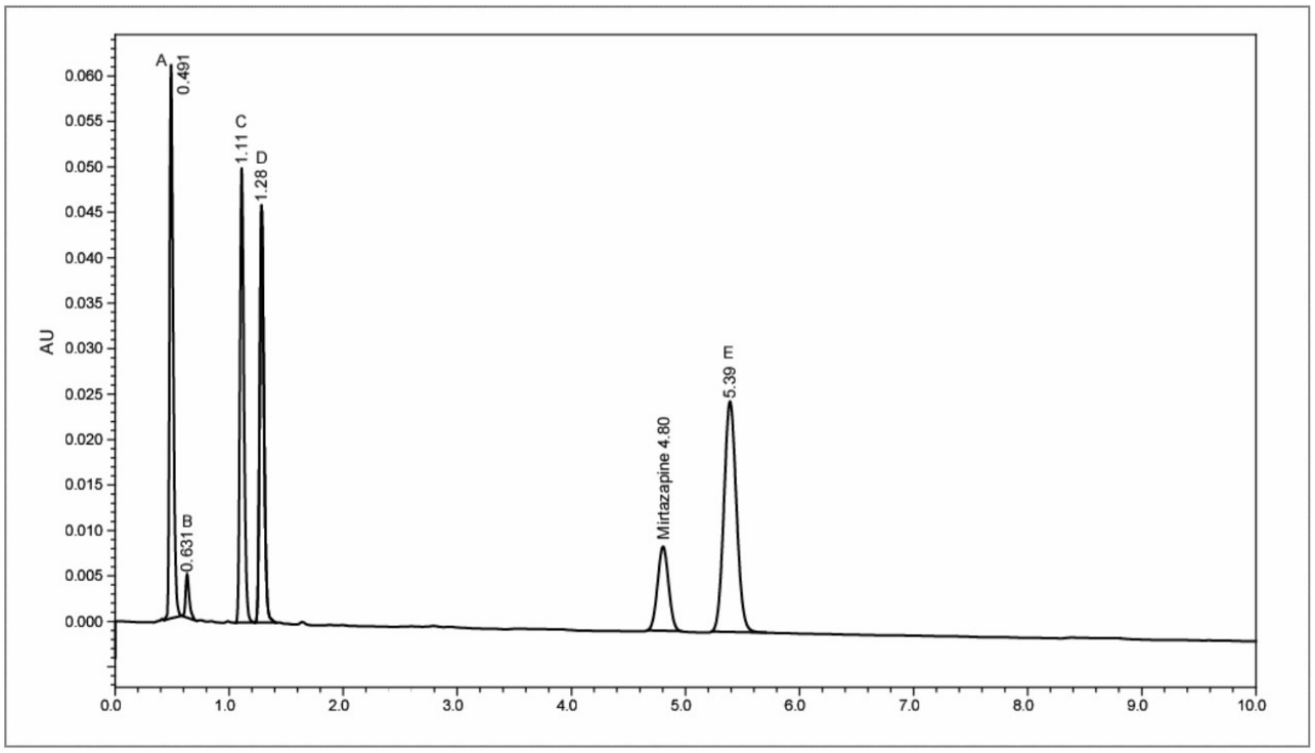


Figure 3. Transferred UPLC method chromatogram (0.10 µL injection).

*Parameter**	*A*	*B*	*C*	*D*	*Mirtazapine*	*E*
Mean RT min.	0.491	0.631	1.11	1.28	4.80	5.39
RRT to Mirt.	0.10	0.13	0.23	0.27	1.0	1.1
USP Resolution	NA	2.45 ± 0.57%	7.90 ± 0.84%	2.62 ± 0.23%	28.2 ± 0.48%	3.05 ± 0.10%
USP Tailing	1.36 ± 0.05%	1.60 ± 0.63%	1.24 ± 0.16%	1.22 ± 0.16%	1.03 ± 0.033%	1.07 ± 0.28%
%RSD Area	0.67	0.74	0.58	0.65	0.88	0.60

**RT = retention time, RRT = relative retention time, Mirt. = mirtazapine*

Table 3. Results from transferred UPLC method [n = 3].

The resolution between the A/B and the C/D peak pairs was >2.0. Tailing factors for each substance were 1.6 or below, and the triplicate area %RSDs were all less than 1%. At this point, the direct method migration from the

USP HPLC method to ACQUITY UPLC method is complete and has resulted in an analysis equivalent in overall performance to the original USP HPLC method (Table 3).

The USP HPLC method was successfully transferred with a minimum of effort to the ACQUITY UPLC System. Simple proportional scaling was all that was employed. Table 4 compares the method parameters of the original USP HPLC and transferred UPLC methods. This simple and direct method transfer yielded an analysis that easily met the chosen acceptance criteria (Table 3). This transferred UPLC method saved 50 minutes per injection while maintaining USP system suitability requirements. As well, the transferred UPLC analysis required 100x less sample injection volume, per injection, than the USP HPLC method and used 97% less volume of mobile phase per run, lowering the costs of both purchase and disposal of mobile phase components. This transfer work demonstrated a simple, straightforward transfer of an USP HPLC method to the ACQUITY UPLC System.

Parameter	*USP HPLC Method*	*Transferred ACQUITY UPLC Method*
Column	4.6 x 250 mm, 5 µm XBridge C_{18}	2.1 x 50 mm, 1.7 µm ACQUITY UPLC BEH C_{18}
Column Temp.	40 °C	40 °C
Mobile Phase	A: Tetramethyl ammonium hydroxide pentahydrate, pH 7.4	A: Tetramethyl ammonium hydroxide pentahydrate, pH 7.4
	B: Acetonitrile:Methanol:THF (43:36:21)	B: Acetonitrile:Methanol:THF (43:36:21)
	Isocratic, 67% A:33% B	Isocratic, 67% A:33% B
Run Time	60 min	10 min
RT Mirtazapine	38.5 min	4.8 min
Flow rate	1.5 mL/min	0.30 mL/min
Injection volume	10 µL	0.1 µL

Table 4. Comparison of USP HPLC and transferred UPLC method parameters.

At this point, the transferred ACQUITY UPLC method can be used as is, or if working in a validated environment, validated using conventional procedures. Although significant gains were achieved from this method transfer, redevelopment to further improve the chromatography was performed to take better advantage of the capabilities of UltraPerformance LC.

Redevelopment

While the migration of the USP HPLC method to UPLC achieved the required USP system suitability requirements, there were a number of reasons to redevelop the method. The complex mobile phase, sub-optimal flow rate (0.30 mL/min compared to the optimal 0.65 mL/min for the 1.7 µm particle column), poor retention and resolution of the A and B related substances pair, as well as a method which was considered to be relatively non-rugged. Experiments showed that even small changes in the organic mixture (< 1% of any of the 3 organic components) could cause the method to fail suitability requirements. As was mentioned in the introduction, the modifications required in the transfer process warranted revalidation. Rather than attempt validation on the transferred method, a redevelopment effort was initiated. The main goals of this method redevelopment were to maintain the suitability requirements outlined in the original USP method, to further reduce run time, and provide a simpler method that would be more rugged than either the original USP HPLC method or the transferred UPLC method. The method development approach was straightforward and will be briefly summarized. Basic compounds, like mirtazapine and its related substances, are known to be effectively separated using high pH mobile phases.

The ACQUITY UPLC BEH columns can operate in these high pH environments. The ammonium bicarbonate buffer was chosen to ensure that the redeveloped method was compatible with mass spectrometry, if needed.

A mix of mirtazapine and its related substances (approx. 1 mg/mL each in 1:1 water/acetonitrile) was analyzed using gradient chromatography on an 2.1 x 50 mm, 1.7 µm ACQUITY BEH C_{18} Column using a 10 mM ammonium bicarbonate buffer (pH 10.5) against acetonitrile. The injection volume was 1.0 µL and the flow rate was 0.65 mL/minute. Five and ten minute preliminary runs were conducted using a 5 to 85% acetonitrile linear gradient. The chromatographic results of these runs were then transferred to DryLab. Subsequent modeling yielded a five minute (including a flow rate increase to 1.2 mL/min) separation method (Table 5) for mirtazapine and its related substances that achieved the method goals. Slight refinements to the gradient starting conditions and slope were then made to the method to further improve resolution: especially between mirtazapine and related substance E. The chromatograms of the original USP HPLC method and the newly developed UPLC method are shown in Figures 4 and 5. This newly developed ACQUITY UPLC method is complete and has resulted in an analysis equivalent or better in overall performance to the original USP HPLC method and the transferred UPLC method (Table 6).

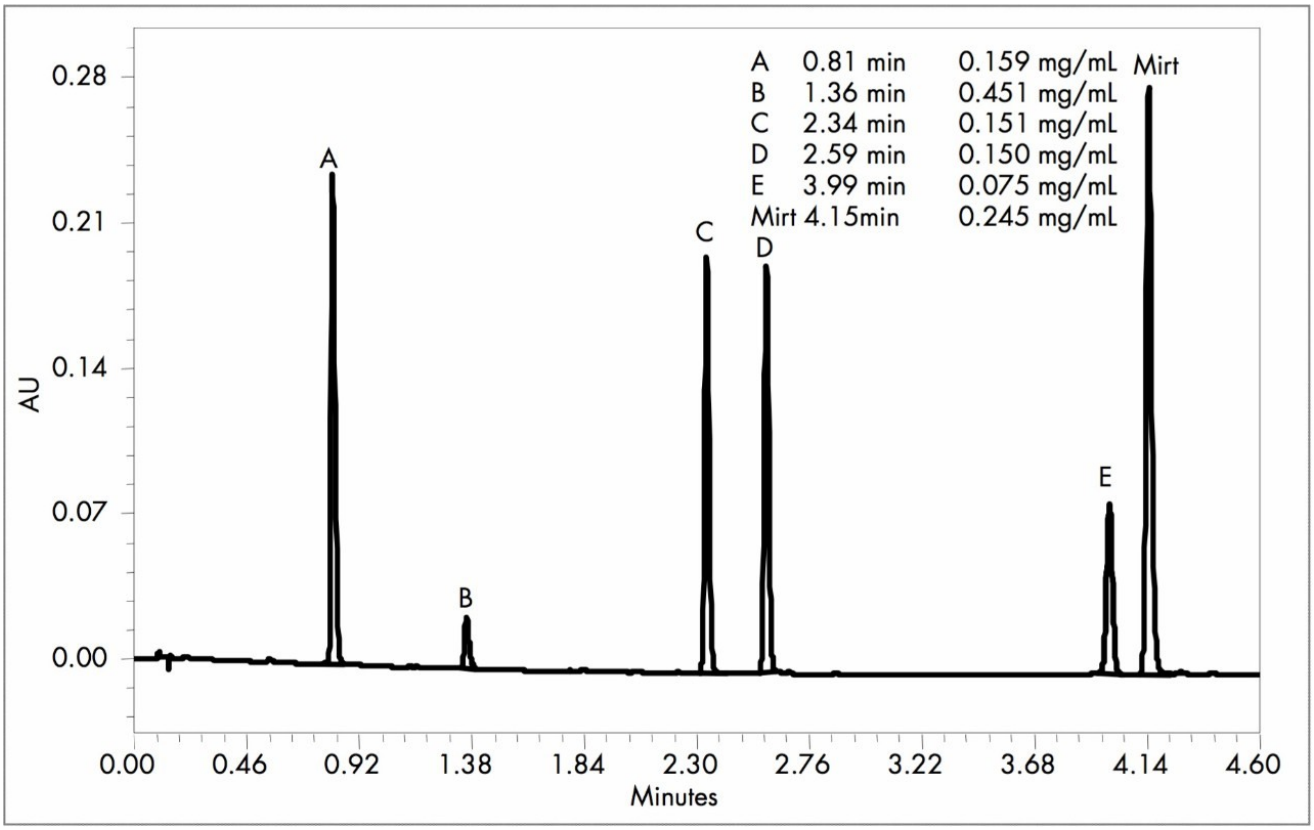


Table 5. Comparison of original USP HPLC and newly developed UPLC method parameters.

Parameter	*Original USP HPLC Method*	*Newly Developed ACQUITY UPLC Method*
Column	4.6 x 250 mm, 5 µm XBridge C_{18}	2.1 x 50 mm, 1.7 µm ACQUITY BEH C_{18}
Column Temp.	40 °C	40 °C
Mobile Phase	A: Tetramethyl ammonium hydroxide pentahydrate, pH 7.4	A: 10 mM Ammonium bicarbonate, pH 10.5
	B: Acetonitrile:Methanol:THF (43:36:21)	B: Acetonitrile
	Isocratic 67% A:33%B	Gradient 5–37% B over 4.5 min
Run Time	60 min	4.6 min.
RT Mirtazapine	26.7 min	4.15 min
Flow rate	1.5 mL/min	1.2 mL/min
Detection	240 nm at 5 pps	240 nm at 20 pps
Injection volume	10 µL	1 µL

Figure 5. Representative redeveloped UPLC method.

*Parameter**	*A*	*B*	*C*	*D*	*E*	*Mirtazapine*
Mean RT min.	0.81 ± 0.12%	1.36 ± 0.18%	2.34 ± 0.11%	2.59 ± 0.11%	3.99 ± 0.06%	4.15 ± 0.07%
RRT	0.20	0.33	0.56	0.62	0.96	1.0
USP Resolution	NA	17.1 ± 0.47%	29.9 ± 0.05%	7.45 ± 0.01%	36.3 ± 0.12%	3.78 ± 0.64%
USP Tailing	1.60 ± 0.01%	1.37 ± 0.80%	1.01 ± 0.02%	1.06 ± 0.00%	1.02 ± 0.09%	1.19 ± 0.20%
%RSD Area	0.10	0.19	0.26	0.27	1.44	0.50

**RT = retention time, RRT = relative retention time*

Table 6. Results from newly developed UPLC method [n = 3].

Conclusion

In this application note, a USP HPLC method for the analysis of mirtazapine and its related substances was first geometrically transferred to a UPLC method. Although this process, and method, was successful, the USP method was totally redeveloped into an improved UPLC method. As can be seen from Table 6, all method goals were easily met by a method that was faster, and provided better resolution of mirtazapine and its related substances, and of the related substances from each other. Initial validation results demonstrated that the new UPLC method could be validated as developed. At 4.5 minutes, the redeveloped UPLC method was much faster that the previous HPLC method (60 minutes), and more than twice as fast as the transferred UPLC method (10 minutes).

Many chromatographers are reluctant to redevelop methods because of the time and effort required to validate a new method. With the significantly reduced run times generated by UPLC (either by geometric transfer or method redevelopment) and the advent of new software products such as the Empower 2 Software Method Validation Manager, method validation has become less time consuming and easier than ever before.

In conclusion, although method migration should be considered when first applying UltraPerformance LC to current or legacy methods, serious consideration should also be given to the redevelopment of these methods to realize the full benefits of this technology. The increases in peak resolution, faster analysis times, and more rugged methods make this a worthwhile endeavor to decrease time to market and lower overall development costs.

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720001866, November 2006