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應用手冊

UPC^2 Method Development for Achiral Impurity Analysis

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Abstract

This application note demonstrates an successful achiral analysis of metoclopramide and related substances

performed using the ACQUITY UPC2 System. The method development process uncovered multiple [stationary phase – analyte] interactions during the comparison of the column screening process. Understanding the influence of these interactions with method variables will help build an appropriate method development approach.

Benefits

- Orthogonal determination of pharmaceutical impurity profiles
- UPC^2 method development approaches for pharmaceutical impurity analysis
- Supercritical fluid chromatography of impurities that meet ICH guidelines and regulatory requirements

Introduction

UltraPerformance Convergence Chromatography (UPC^2) exploits the benefits of a sub-2-µm particle size stationary phase with carbon dioxide as the primary mobile phase component. Convergence chromatography is an analytical tool that uses less solvent, yet provides high speed separations. Particularly for impurity analysis, convergence chromatography provides an orthogonal approach aiding discovery of unknown impurities when compared to reversed phase LC. While method development strategies in liquid and gas chromatography are well-defined, that is not the case for convergence chromatography. In order to streamline this process, a systematic approach to achiral convergence chromatography method development requires research.

It is important to understand the impurity profiles of drug products and drug substance material. Assessing the purity of the sample allows pharmaceutical companies to make decisions during the development and to move forward through commercialization of the drug. Impurity profiles dictate raw material quality from vendors, finished product shelf life, route synthesis pathways, and intellectual protection from counterfeiting. Orthogonal comparisons of the chromatography profiles provide the ability to make the best educated decisions. In this application, the ACQUITY UPC^2 System was used to analyze metoclopramide and related impurities. Metoclopramide, as shown in Figure 1, is an antiemetic drug used for the treatment of heartburn, healing of ulcers, and nausea resulting from chemotherapy. The method development investigated columns and solvents to determine suitable method conditions optimizing specificity and peak shape.

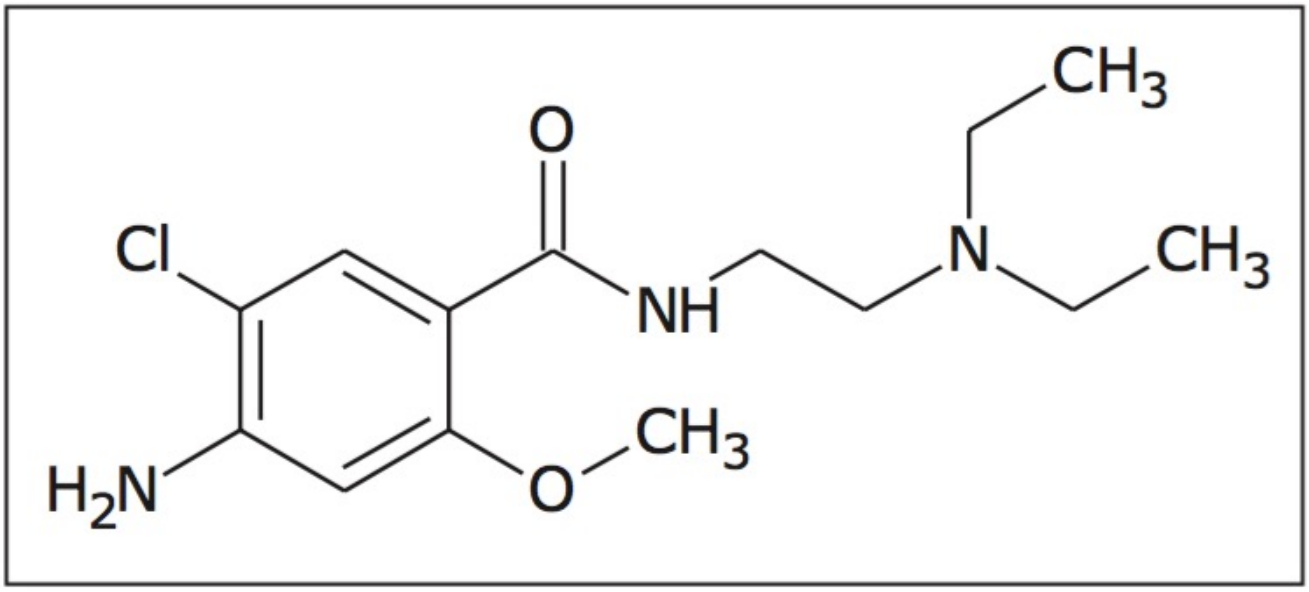

Figure 1. Chemical structure of metoclopramide.

Experimental

UPC^2 conditions	
System:	ACQUITY UPC^2 with PDA and SQD detection
Column:	ACQUITY UPC^2 BEH 2-EP 3.0 x 100 mm, 1.7 µm
Mobile phase A:	CO_2
Mobile phase B:	1 g/L Ammonium formate in 50:50 methanol/acetonitrile spiked with 2% formic acid
Wash solvents:	70:30 Methanol/ isopropanol
Separation mode:	Gradient; 2% to 30% B over 5.0 min; held at 30% for 1 min
Flow rate:	2.0 mL/min

UPC^2 conditions	
CCM Back Pressure:	1500 psi
Column temp.:	50 °C
Sample temp.:	10 °C
Injection volume:	1.0 µL
Run time:	6.0 min
Detection:	PDA 3D Channel: PDA, 200 to 410 nm; 20Hz PDA 2D Channel: 270 nm at 4.8 nm Resolution (compensated 500 to 600 nm) SQD MS: 150 to 1200 Da; ESi+ and ESi-
Make-up flow:	None required
Data management:	Empower 3 Software

Sample description

A resolution solution was prepared with metoclopramide and eight related impurities, then placed in a TruView Maximum Recovery Vial for injection, as shown in Table 1. The impurities were prepared at 0.1% w/w concentration of the metoclopramide standard. The resolution solution was used for the chromatographic method development.

Peak #	Name	FW	EP ref.
	METOCLOPRAMIDE (4-amino-5-chloro-N-(2-(diethylamino)ethyl)-2-methoxybenzamide	299.8	
1	4-amino-5-chloro-2-methoxybenzoic acid	201.6	(EP C)
2	4-(acetylamino)-2-hydroxybenzoic acid	195.2	(EP H)
3	4-amino-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide N-oxide	315.8	(EP G)
4	4-amino-5-chloro-N-2-(diethylaminoethyl)-2-hydroxybenzamide	285.8	(EP F)
5	4-(acetylamino)-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide	341.8	(EP A)
6	Methyl 4-(acetylamino)-2-methoxybenzoate	223.2	(EP D)
8	Methyl 4-(acetylamino)-5-chloro-2-methoxybenzoate	257.7	(EP B)
9	Methyl 4-amino-2-methoxybenzoate	181.1	

Table 1. List of metoclopramide impurity standards, peak designation, masses, and European Pharmacopoeia labels.

Results and Discussion

Systematic screening

The method development process systematically screened columns, modifiers, and modifier additives to achieve the best separation. The initial configuration screened four UPC^2 columns with four modifiers. A "modifier" is the strong solvent mobile phase that facilitates elution of the analytes increasing in polarity. The four solvents used were methanol, methanol with 0.5% formic acid, methanol with 2 g/L ammonium formate, and methanol with 0.5% triethylamine. The screening process was performed with a generic 5% to 30% B gradient over 5 min, holding at 30% for 1 min. The total screening time was achieved in just over two hours. The methanol with ammonium formate provided the best overall peak shape compared for each column, as shown in Figure 2. The peak tracking during the method screening process was achieved by reviewing the MS spectra provided by the ACQUITY SQD. The selectivity (α) for the more polar analytes varied greatly. Since the mobile phase was held constant for these comparisons, the result of changing α are due to [stationary phase – solute] interactions.

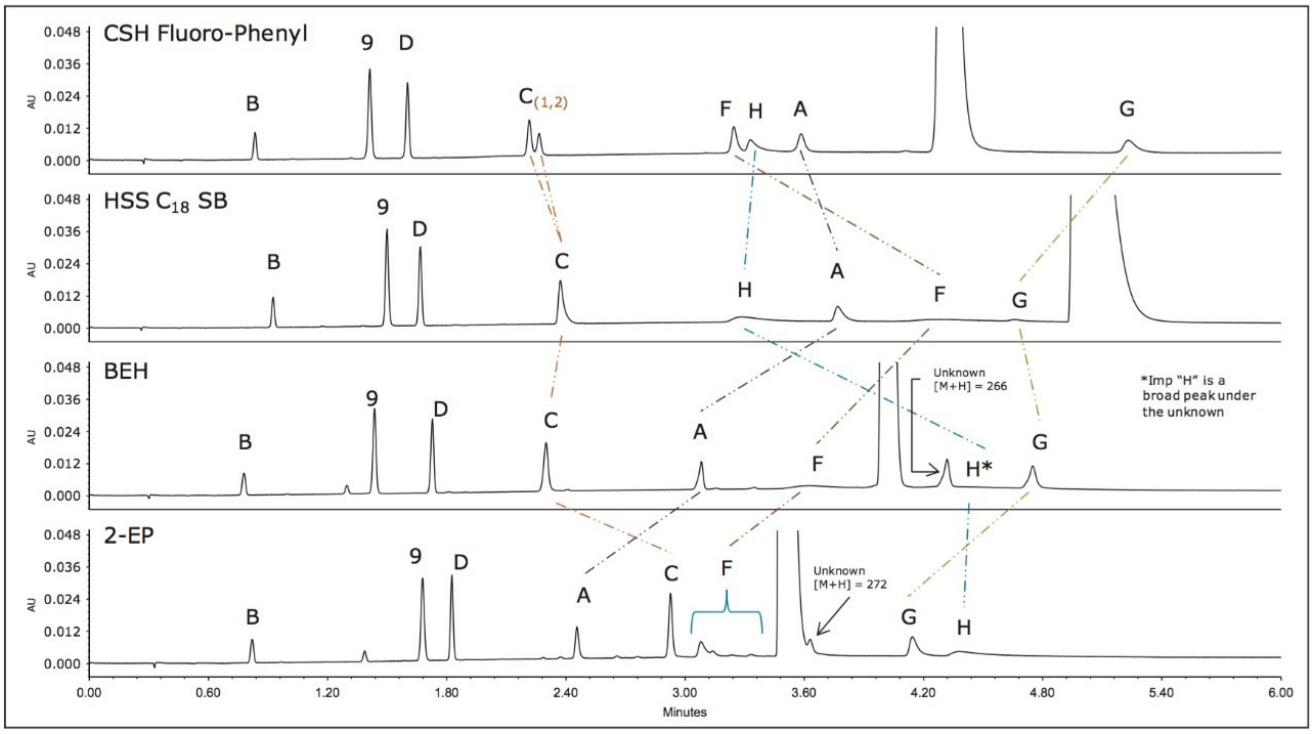

Figure 2. Column screening results. The modifier (B) was methanol with 2 g/L ammonium formate. 5% to 30% B over 5 min and held at 30% for 1 min.

Based on the results, the UPC^2 2-EP stationary phase was the optimal column of choice providing better peak shape and resolution for the majority of the analytes. The UPC^2 CSH Fluoro-Phenyl column provided good selectivity and peak shape; however, impurity C unexpectedly separated into two peaks. This unknown phenomenon will be explored in another set of experiments outside the scope of this application note.[1]

Effect of gradient slope

In reversed phase LC, gradient slope is a common tool to manipulate selectivity and resolution. Using the UPC^2 2-EP stationary phase, the gradient slope was decreased by extending the overall gradient run time. The change in slope had little to no effect on the chromatographic profile with the exception of a selectivity change between peaks 6 and 7, as shown in Figure 3.

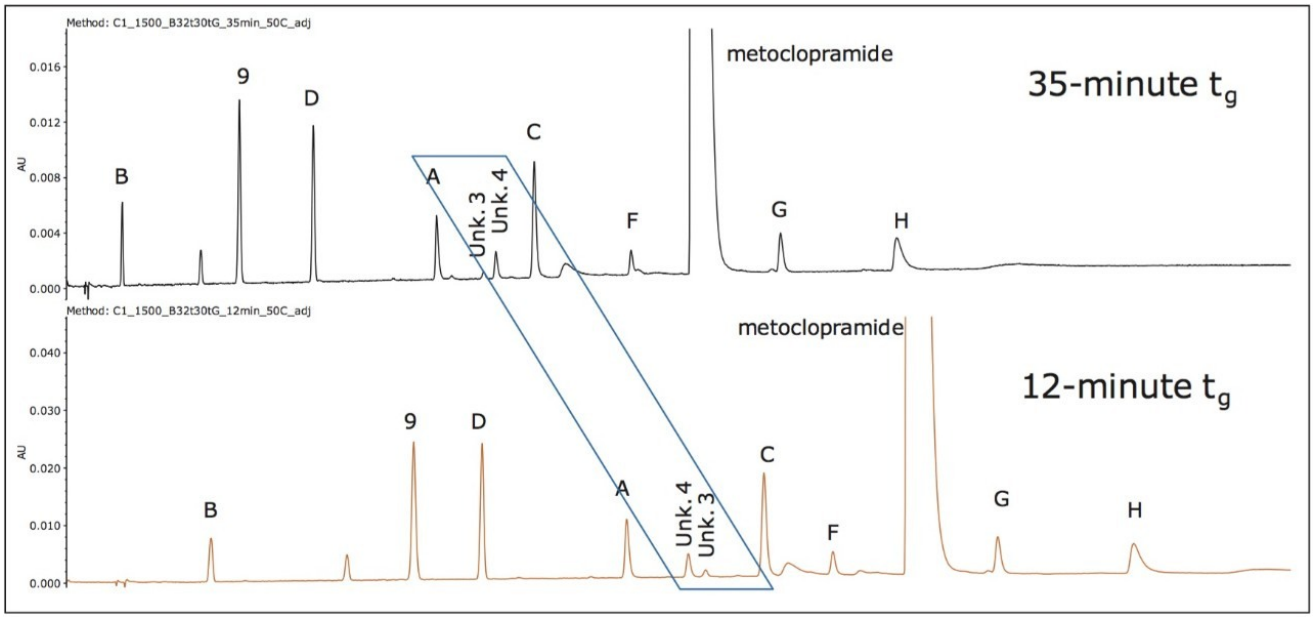

Figure 3. Normalized x-axis overlay metoclopramide analyzed with extended 12- and 35-minute gradient run times flattening the slope compared to the 6-minute screening experiments. The original gradient was used; 5% to 30% B.

Effect of different elution solvents

Inducing a shallower gradient did not increase resolution between peaks. To increase resolution, a less polar aprotic organic solvent (acetonitrile) was mixed at different compositions with methanol, the stronger elution solvent. The addition of acetonitrile increased resolution, spreading the separation space. Based on these observations, this technique proves to be a powerful tool when developing methods, as observed in previously published results.[1]

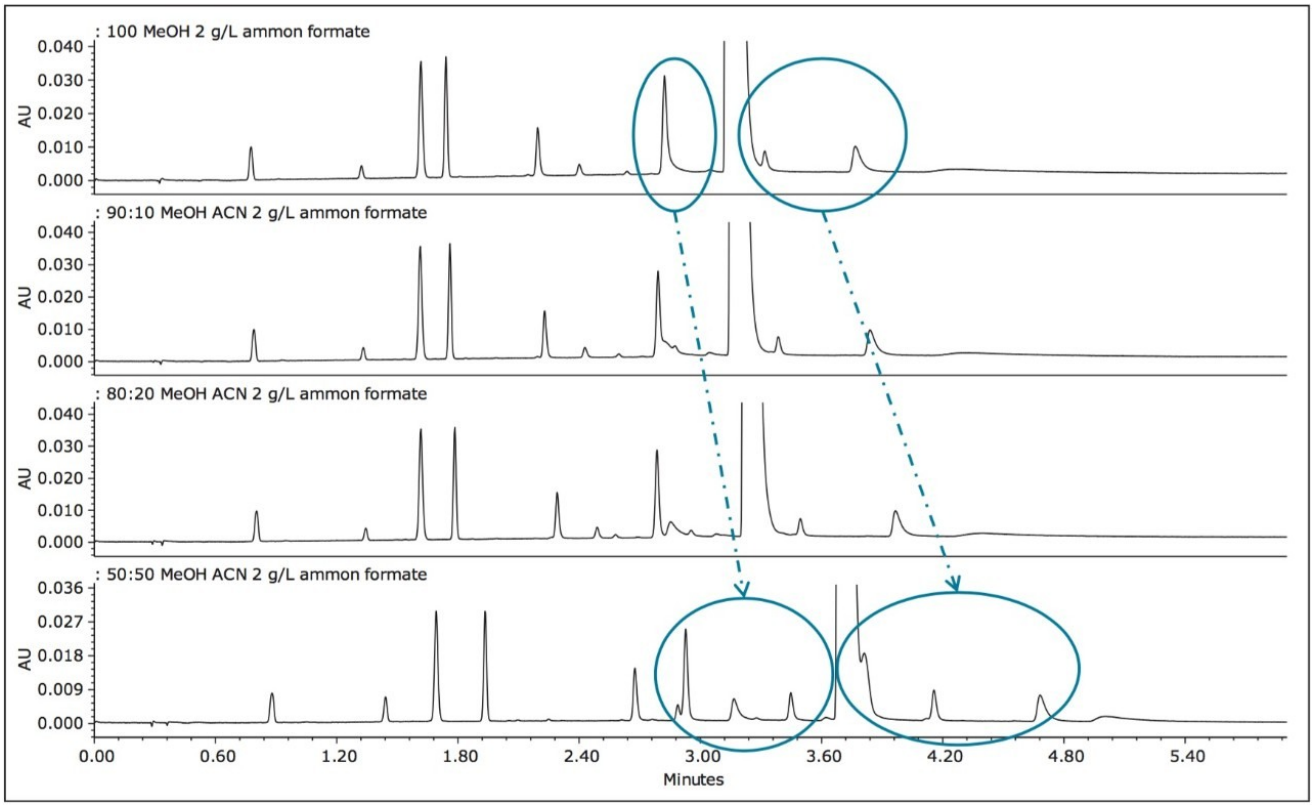

Figure 4. Highlighted in this overlay, the addition of acetonitrile to the composition of the modifier increased the resolution of the later eluting analytes.

Effect of Additive

The effect of additives can either enhance or mask analyte interactions with the stationary phase. The metoclopramide impurities have many different side group functionalities. The related impurities, shown in Table 1, indicate amines, carboxylic acids, and hydroxyl groups. Therefore, choosing a suitable additive is challenging. Ammonium formate improved peak shapes for many of the compounds in the mixture. The other additives explored during the screening process improved the peak shape of other analytes, such as impurity H; however, it affected the other components in the mixture.

Individual standards of each of the impurities were explored during the additives screening. Formic acid achieved acceptable peak shape for impurity H; however, the chromatographic performance of the other related substances were affected. The effect of additive concentration can influence peak shape. Concentrations greater than those usually practiced with reversed phase LC may be required to achieve desired peak shape. Increasing the concentration of the formic acid yielded further improvements in peak shape for impurity H, as shown in Figure 5. Unfortunately, the peak shape for impurity F was compromised, as seen in Figure 6. Combining formic acid and ammonium formate provided the benefits of each additive, resulting in optimal peak shape for the entire

separation. The results of the formic acid, ammonium formate, and combination of additives in the modifier for the expired sample are shown in Figure 7. By using the expired sample in this comparison, we can better assess the selectivity and peak shape effects of the known impurities in the presence of the unknown impurities. As seen in Figure 7, addressing peak shape ultimately affects the efficiency, resolution, and sensitivity of the chromatographic separation.

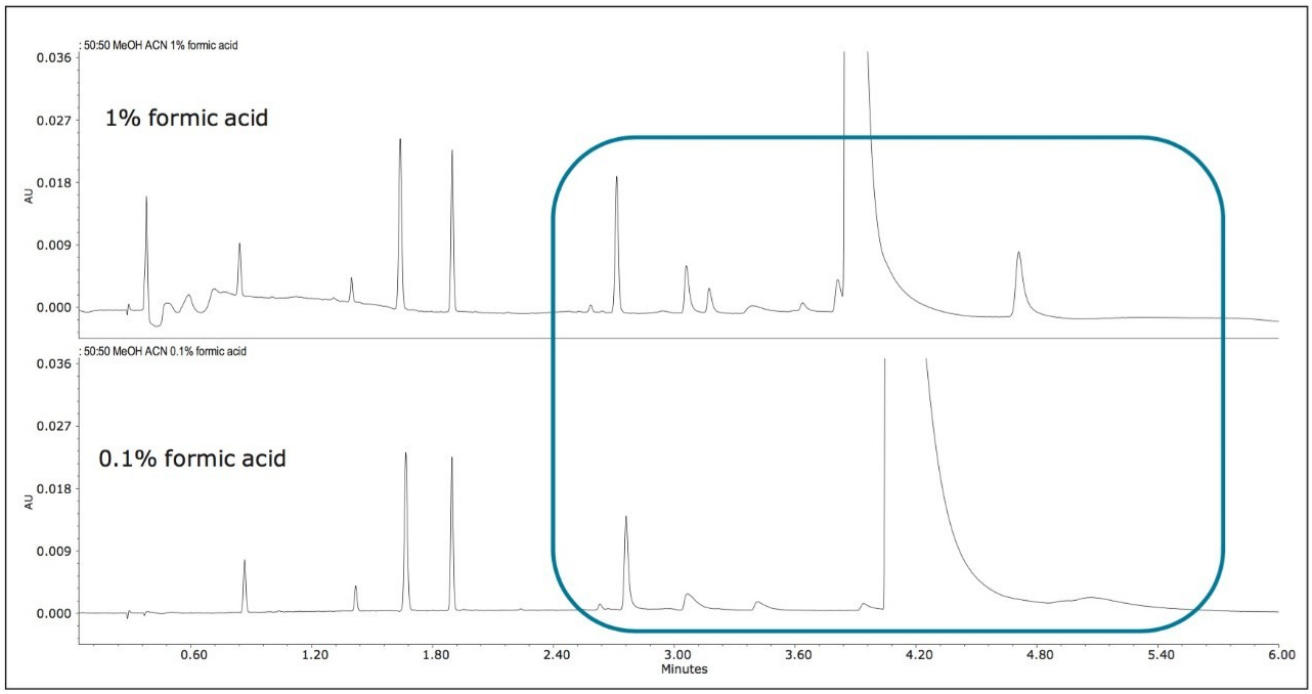

Figure 5. Comparisons of two modifiers; 0.1% formic acid in methanol versus 1% formic acid in methanol. The polar analytes (highlighted in the box) improved with increases in additive concentration, while the more neutral components were not affected. (Refer to the Experimental section for additional method parameters.)

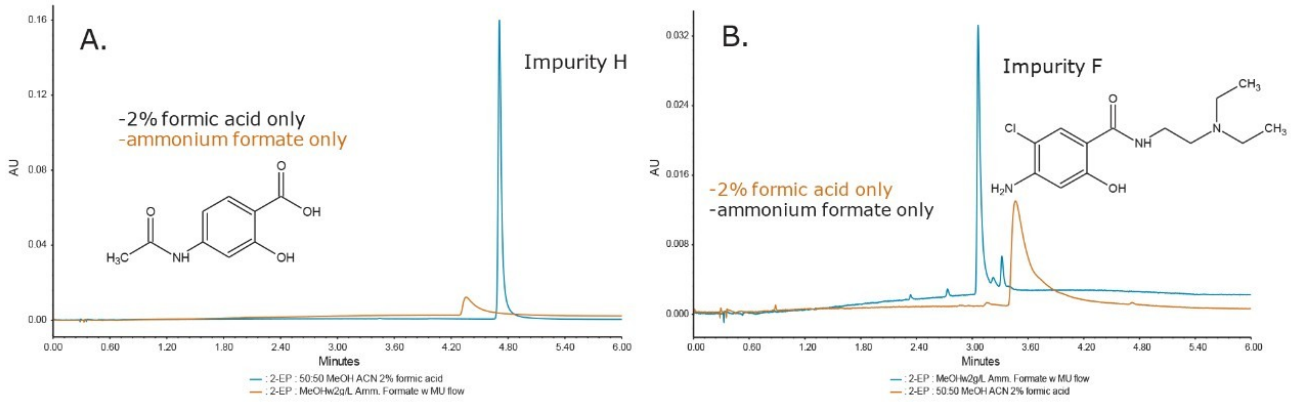

Figure 6. Peaks with hydroxyl (or polyphenols) functionality such as impurity H tend to benefit from the use of only formic acid, as shown in Figure 5A. Optimal peak shape for compounds with primary, secondary, and tertiary amine functionality trend from the use of ammonium salt-based additives as with impurity F, as shown in Figure 5B.

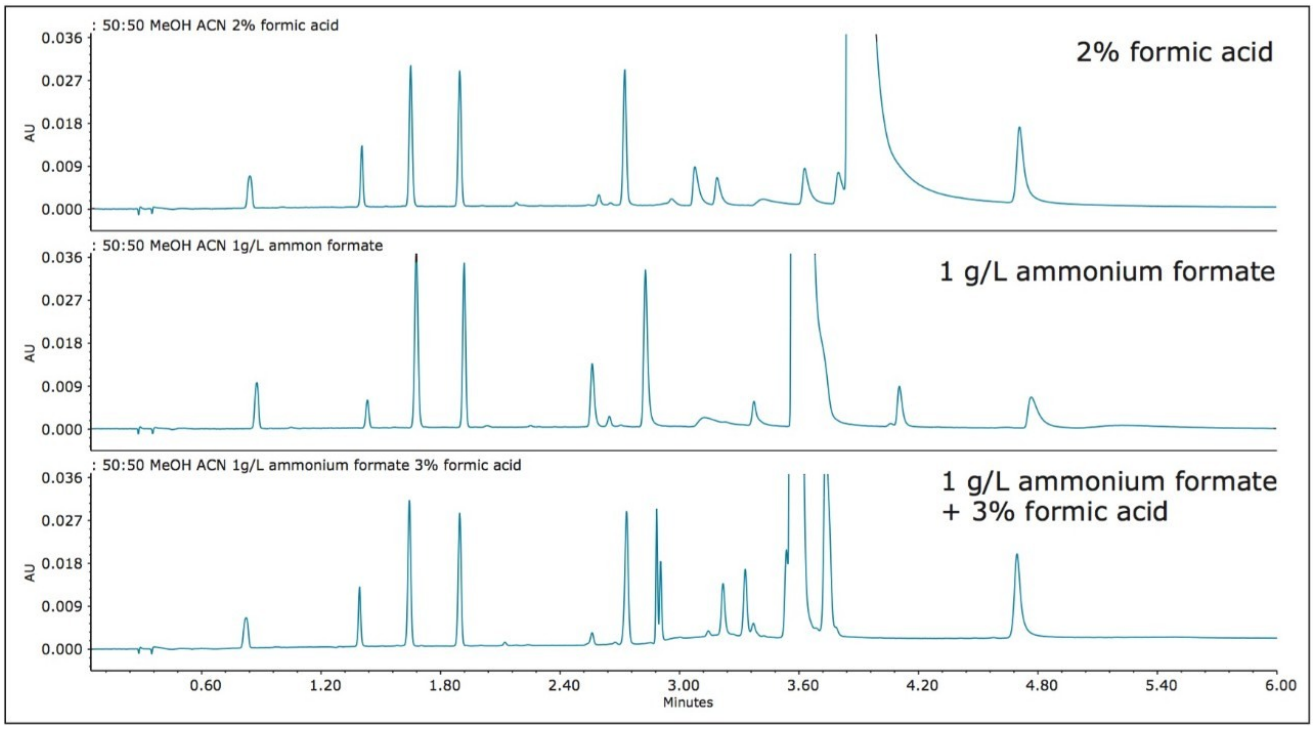

Figure 7. Injections of an expired metoclopramide sample performed with different additive compositions in the modifier. Combining ammonium formate with formic acid in terms of a "buffer-like" system provided the best peak shape for all analytes in the sample. The modifier used was 50:50 methanol/acetonitrile.

Evaluating Specificity

Once the method conditions that positively influenced selectivity, resolution, and peak shape were determined, variables were optimized. The final method was evaluated with a standard mixture of metoclopramide and

impurities (control) and an expired sample mixture, as shown in Figure 8. Further interrogations of the unknown impurities are addressed in a Waters application note.[2]

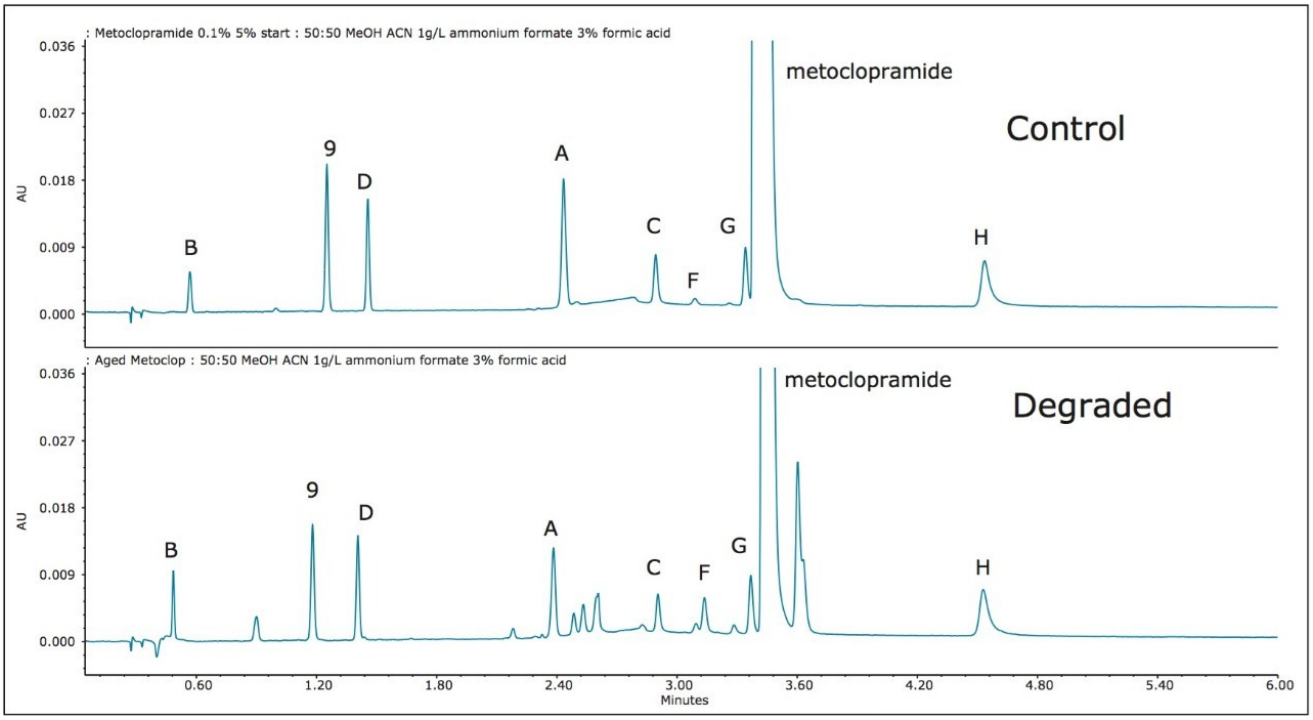

Figure 8. Comparison of a metoclopramide control mixture and a degraded mixture using the final method conditions outlined in the Experimental section.

Conclusion

An achiral analysis of metoclopramide and related substances was successfully performed using the ACQUITY UPC^2 System. The method development was facilitated by understanding the properties of the impurity structures. Many of the impurities consisted of amines, hydroxyl groups, esters, and carboxylic acids. The primary method variables that influenced selectivity, resolution, and peak integrity were stationary phase, modifier elution strength, and additive composition, respectively. The final metoclopramide related substances method demonstrated specificity for an expired metoclopramide sample.

The method development process uncovered multiple [stationary phase – analyte] interactions during the comparison of the column screening process. Further research, in addition to and guided by those previously published,[3-6] need to be explored. Understanding the influence of these interactions with method variables will help build an appropriate method development approach.

References

1. Jones MD, ***et al.*** Analysis of Organic Light Emitting Diode Materials by UltraPerformance Convergence Chromatography Coupled with Mass Spectrometry (UPC^2/MS). Waters Application Note 720004305EN. 2012 April.
2. Jones MD, ***et al***. Impurity Profiling Using UPC^2/MS. Waters Application Note 720004575EN. 2013 Jan.
3. West C, Lesellier E. A unified classification of stationary phases for packed column supercritical fluid chromatography. *J Chromatogr A*. 2008 May; 1191(1-2):21-39.
4. West C, Khater S, Lesellier E. Characterization and use of hydrophilic interaction liquid chromatography type stationary phases in supercritical fluid chromatography. *J Chromatogr A*. 2012 Aug; 1250:182-95.
5. Lesellier E. Retention mechanisms in super/subcritical fluid chromatography on packed columns. *J Chromatogr A*. 2009 Mar; 1216(10):1881-90.
6. Zou W, Dorsey JG, Chester TL. Modifier effects on column efficiency in packed-column supercritical fluid chromatography. *Anal Chem*. 2000 Aug; 72(15):3620-6.

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