

## Improving Effectiveness in Method Development by Using a Systematic Screening Protocol

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Margaret Maziarz, Sean M. McCarthy, Mark Wrona

Waters Corporation

### Abstract

In this application, we present the development of a UPLC method, using the USP-defined assay for metoclopramide HCl and related substances as an example. Using the ACQUITY QDa Detector in conjunction with UV detection and the ACQUITY UPLC H-Class System streamlined the method development process. We demonstrate:

- Robust UPLC method development
- Quick and accurate identification of sample components using mass detection with the ACQUITY QDa Detector
- Minimize the need for running individual injections of sample components to confirm the identity of peaks

A systematic protocol is employed that includes scouting, screening, and optimization steps. Results for each step are analyzed and ranked using custom calculations and reported within Empower 3 Chromatography Data Software to minimize analyst bias in decision making and ensure the overall goals are achieved.

The criteria for success with a goal of separating all nine components, achieving a resolution of  $\geq 2.0$ , tailing of  $\leq 1.5$ , and retention factor ( $k^*$ )  $\geq 3.0$ , were met.

Finally, the use of ApexTrack in Empower Software enabled consistent evaluation of chromatograms for fair

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comparison across the development process. Empower custom calculations and reporting allowed us to generate a scoring report to easily identify the best conditions at each step in our protocol.

Overall, using a defined systematic protocol with the UPLC system, detectors, and its column chemistries enables analytical laboratories to quickly and efficiently develop chromatographic methods. Methods developed in this manner are typically more reproducible, which allows laboratories to have a higher validation success rate.

## Benefits

- Robust UPLC method development
- Quick and accurate identification of sample components using mass detection with the ACQUITY QDa Detector
- Minimize the need for running individual injections of sample components to confirm the identity of peaks

## Introduction

Method development involves screening a range of chromatographic parameters to generate sufficient resolution and robust separations. While there are many approaches to method development, such as one factor at a time, systematic, and quality by design (QbD), the goals and factors used for optimizing separations are the same. The parameters that are adjusted include column chemistry, organic solvent, pH, gradient slope, flow rate, temperature, among other factors.

The impact of modifying these parameters are then systematically evaluated during development. Methods from each round of optimization are assessed using specific criteria such as the greatest number of peaks of interest with appropriate retention, resolution, and tailing values. The best method(s) from each step are then selected for further investigation until a suitable method is obtained. Throughout this development process, it is essential to ensure selection of the best conditions at each step and have demonstrable reasons for selection.

Regardless of the optimization strategy selected, it is important to identify and track critical sample components across the conditions investigated. Because peak elution order can change and UV spectra of related substances can be indistinguishable, standards (if available) are sequentially injected under the same conditions to simplify analysis. While ultimately effective, this is a time-consuming process. Using mass detection in addition to optical detection enables unambiguous identification. It also enables analysts to monitor sample components, and to

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rapidly identify and track coelutions and elution order changes.

In this application, we present the development of a UPLC method for metoclopramide HCl and related substances. We combine UV (PDA) and mass detection, with the user-friendly ACQUITY QDa Detector. A systematic protocol is employed that includes scouting, screening, and optimization steps. Results for each step are analyzed and ranked using custom calculations and reported within Empower 3 Chromatography Data Software to minimize analyst bias in decision making and ensure the overall goals are achieved.

## Experimental

### Waters reference standard

Packaged in a vial: LCMS Quality Control Reference Material (QC-Reference Material)

### Method development conditions

|                   |  |
|-------------------|--|
| LC system:        | ACQUITY UPLC H-Class with Column Manager and Solvent Select Valve (SSV)  |
| Columns:          | All columns with dimension of 2.1 x 50 mm:<br>ACQUITY UPLC CSH C <sub>18</sub> , 1.7 μm<br>ACQUITY UPLC CORTECS C <sub>18+</sub> , 1.6 μm<br>ACQUITY UPLC CSH Phenyl Hexyl, 1.7 μm<br>ACQUITY UPLC HSS Pentafluorophenyl (PFP), 1.8 μm |
| Column temp.:     | 40, 45, and 50 °C  |
| Injection volume: | 1.0 μL   |
| Flow rate:        | 0.6 mL/min   |
| Mobile phase A:   | 125 mM Formic acid in water  |

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|                  |                                    |
|------------------|------------------------------------|
| Mobile phase B:  | 125 mM Ammonium hydroxide in water |
| Mobile phase C:  | Water                              |
| Mobile phase D1: | Acetonitrile                       |
| Mobile phase D2: | Methanol                           |

## System control, data acquisition, and analysis

|                    |   |
|--------------------|---|
| Data acquisition:  | Empower 3 FR2 CDS Software                                  |
| Separation:        | Standard gradient with 5-90% organic solvent over 5 minutes |
| Wash solvents:     | Purge/Sample Wash: 50:50 water/methanol                     |
| Seal wash:         | 90:10 water/acetonitrile                                    |
| PDA detector:      | ACQUITY UPLC PDA  |
| PDA settings:      | 210-400 nm (derived at 270 nm)                              |
| MS detector:       | ACQUITY QDa (Extended Performance)                          |
| Scan mode:         | 100-400 <i>m/z</i>  |
| Ionization mode:   | ESI+, ESI-  |
| Probe temp.:       | 600 °C  |
| Sampling rate:     | 10 pts/sec  |
| Capillary voltage: | 0.8 kV (pos/neg)  |

Cone voltage: 15 V

Data: Centroid

In this application, we demonstrate how using both UV and mass data allows accurate tracking of all components during development and ensures peak purity in the final method. Overall, following a systematic protocol and utilizing mass detection enables faster and more effective development of a chromatographic method that conforms to the USP standard methodology for robustness and performance verification.<sup>1</sup>

## Preparation of Solutions

### Sample solution with APIs and related compounds

Separate stock solutions were prepared in methanol at 1.0 mg/mL. An equal volume of each stock solution was transferred to one vial and diluted with water to make a working sample with a final concentration of 0.06 mg/mL of each analyte. The compounds used in this study are listed in Table 1.

| Compound | Common Name   | Monoisotopic Mass (Da) |
|----------|---|------------------------|
| API      | Metoclopramide  | 299.14                 |
| Imp. A   | 4-Acetamido-5-chloro-N-(2-(diethylamino)ethyl)-2-methoxybenzamide   | 341.15                 |
| Imp. B   | Methyl 4-acetamido-5-chloro-2-methoxybenzamide                      | 257.05                 |
| Imp. C   | 4-Amino-5-chloro-2-methoxybenzoic acid                              | 201.02                 |
| Imp. D   | Methyl 4-acetamido-2-methoxybenzoate                                | 223.08                 |
| Imp. F   | 4-Amino-5-chloro-N-(2-(hydroxybenzamido)-2-hydroxybenzamide         |                        |
| Imp. G   | 2-(4-Amino-5-chloro-2-hydroxybenzamido)-N,N-diethylethanamide oxide | 315.14                 |
| Imp. H   | 4-Acetamido-2-hydroxybenzoic acid                                   | 195.05                 |
| Imp. 9   | Methyl 4-amino-2-methoxybenzoate                                    | 181.07                 |

Table 1. List of USP specified related substances of metoclopramide HCl for UPLC method development.

## Results and Discussion

### Method development systematic protocol

Using a systematic protocol enables a consistent evaluation of major selectivity parameters, which ensures the development of robust and reproducible methods; here, using UPLC for faster and more sensitive analysis.

Column chemistries with different base particles and ligands were selected to reflect a wide selectivity range.

As shown in Figure 1, the protocol is built around a series of steps, each designed to address resolution systematically. The first step in our protocol involves defining our sample, success criteria, chromatographic system, and verifying system performance.

For metoclopramide and its USP-defined related substances, our goal was to separate these components to

achieve a minimum USP resolution of  $\geq 2.0$  for each peak with a USP tailing of  $\leq 1.5$ , and a retention factor ( $k^*$ )  $\geq 3.0$ . The retention factor of a peak for gradient separations is defined as  $k/(k+1)$ .

For the greatest flexibility in development, we used the ACQUITY UPLC H-Class System configured with a Column Manager and Solvent Select Valve. To identify all components and possible coelutions, we used both ACQUITY PDA for optical detection and ACQUITY QDa for mass detection. We verified system performance using a LCMS Quality Control Reference Material to confirm system was operating properly prior initiating the study.<sup>3</sup>

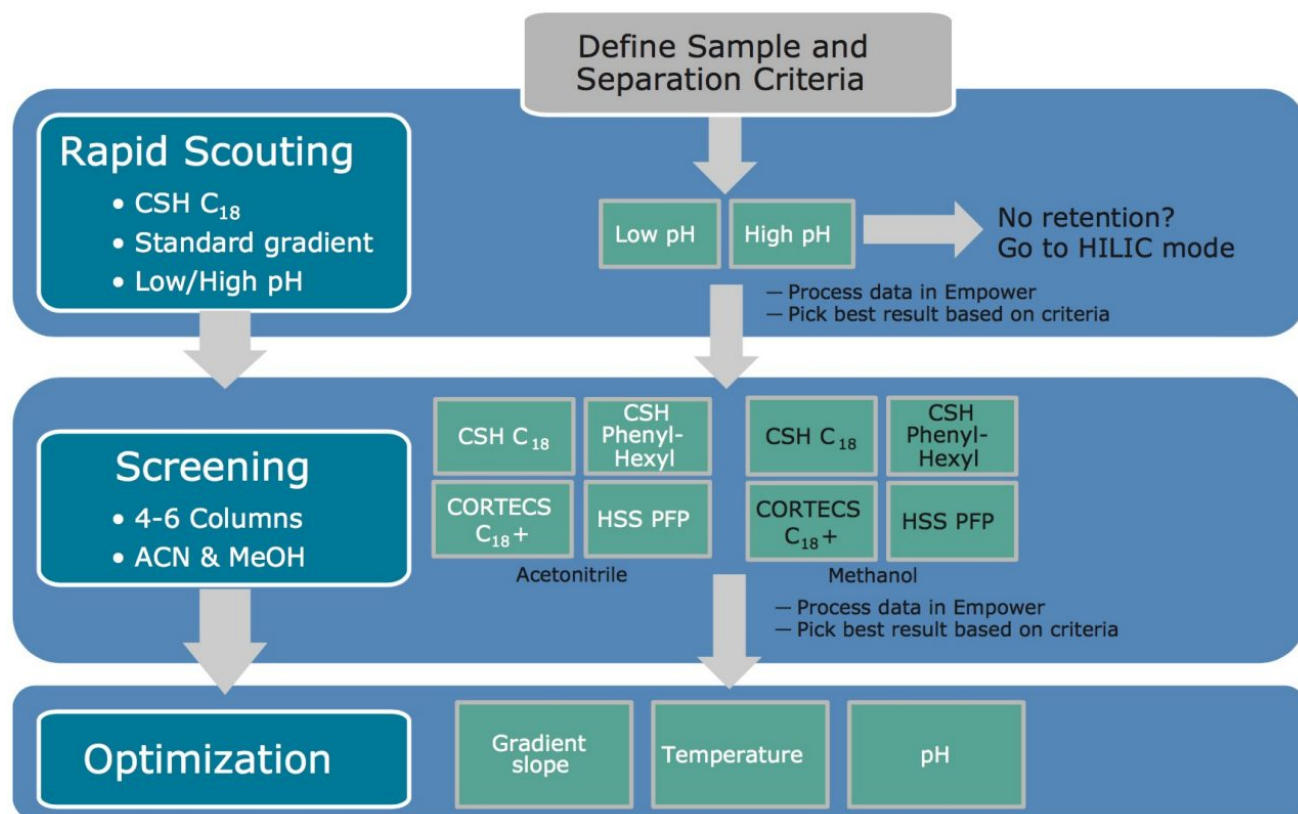


Figure 1. Systematic protocol for development of chromatographic methods.

### Rapid scouting

After defining our sample, criteria, and system, we began the systematic protocol with rapid scouting to quickly screen for an acceptable separation condition. The goal of rapid scouting is to select acidic or basic conditions that provide the best retention of the sample components, as well as to identify the best separation mode (reversed-phase or HILIC).

Low and high pH separations were performed using stock solutions of 125 mM formic acid and 125 mM ammonium hydroxide, respectively. For the reversed-phase separation, we used a standard gradient of 5–90% of

acetonitrile over 5 minutes. As expected for this basic sample mixture, there were dramatic changes in retention observed between the low and high pH separations (Figure 2). We were also able to track which components are most affected by the pH using the mass data. The chromatographic data was processed in Empower automatically using ApexTrack integration to detect peaks.

To determine the best conditions to move forward, we defined custom calculations and created a customized report in Empower Software. The methods were scored and selected using the best conditions by tracking the number of peaks that meet our defined goals. In this case, the best retention of all components is achieved at low pH, and for this reason, we continued our study with low pH.

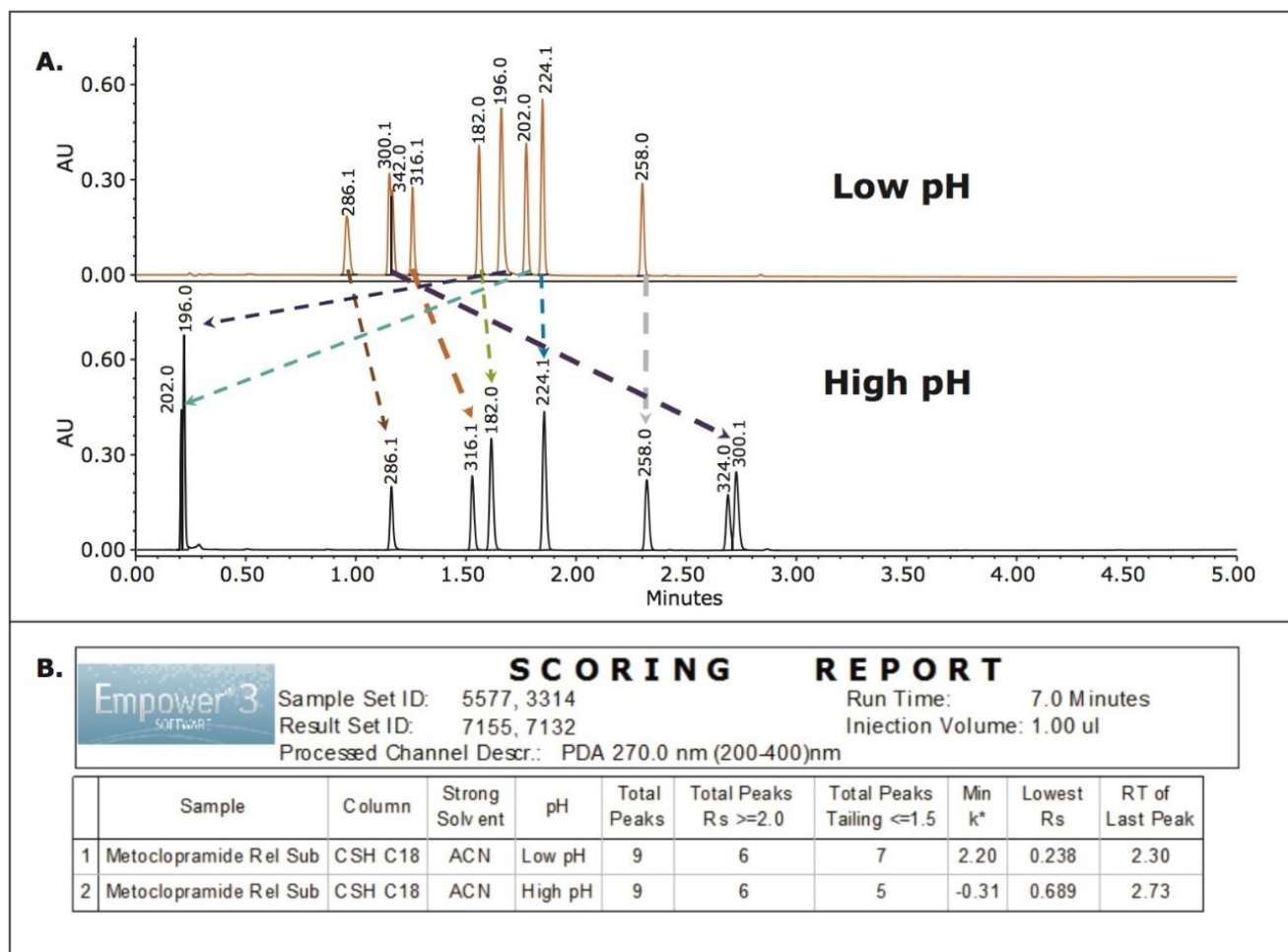


Figure 2. Rapid scouting with low and high pH. A. Chromatographic data showing impact of low and high pH on the separation of metoclopramide and related compounds. The sample components that are most affected by the pH were tracked using the mass data. B. Empower 3 scoring report. Criteria for success were defined in Empower as custom calculations, which were then used to create a report. Criteria were ranked so that best method appears first.



## Screening

The conditions with best retention selected in the scouting step (low pH condition) did not fully meet our criteria for success. We moved to the screening phase of the protocol with a goal of separating all sample components. Using the Column Manager allowed us to select each column without the need for user intervention. For each separation we used the same standard gradient as in the scouting experiments, but investigated both methanol and acetonitrile eluents.

Again, we used the Empower scoring report to analyze the chromatographic data and select the best separation (Figure 3). As shown, the ACQUITY UPLC CSH C<sub>18</sub> Column with methanol provides the highest number of peaks and has the highest number of peaks with resolution  $\geq 2.0$  and a tailing  $\leq 1.5$ . For this reason we selected this condition for the final phase of the systematic protocol, optimization.

| Empower 3 SOFTWARE |                        | SCORING REPORT      |                           | Sample Set ID: 4001                                | Run Time: 7.0 Minutes     |                                |           |        |                 |      |
|--------------------|------------------------|---------------------|---------------------------|--|---------------------------|--------------------------------|-----------|--------|-----------------|------|
|                    |                        | Result Set ID: 7073 | Injection Volume: 1.00 ul | Processed Channel Descr.: PDA 270.0 nm (200-400)nm |                           |                                |           |        |                 |      |
| Sample             | Column                 | Strong Solvent      | pH                        | Total Peaks  | Total Peaks Rs $\geq 2.0$ | Total Peaks Tailing $\leq 1.5$ | Lowest Rs | Min k* | RT of Last Peak |      |
| 1                  | Metoclopramide Rel Sub | CSH C18             | MeOH                      | Low pH   | 9                         | 7                              | 7         | 1.283  | 3.22            | 3.11 |
| 2                  | Metoclopramide Rel Sub | CORTECS C18+        | ACN                       | Low pH   | 9                         | 7                              | 5         | 0.769  | 1.98            | 2.15 |
| 3                  | Metoclopramide Rel Sub | CSH C18             | ACN                       | Low pH   | 9                         | 5                              | 7         | 2.308  | 2.15            | 2.30 |
| 4                  | Metoclopramide Rel Sub | CORTECS C18+        | MeOH                      | Low pH   | 8                         | 7                              | 3         | 2.094  | 2.99            | 2.98 |
| 5                  | Metoclopramide Rel Sub | CSH Phenyl Hexyl    | MeOH                      | Low pH   | 8                         | 6                              | 8         | 1.690  | 2.30            | 3.13 |
| 6                  | Metoclopramide Rel Sub | CSH Phenyl Hexyl    | ACN                       | Low pH   | 8                         | 5                              | 5         | 0.654  | 0.98            | 2.21 |
| 7                  | Metoclopramide Rel Sub | HSS PFP             | MeOH                      | Low pH   | 8                         | 2                              | 2         | 1.870  | 6.86            | 3.44 |
| 8                  | Metoclopramide Rel Sub | HSS PFP             | ACN                       | Low pH   | 7                         | 2                              | 2         | 0.108  | 4.51            | 2.61 |

Figure 3. Empower 3 scoring report for screening different columns and organic solvents. The method using the ACQUITY UPLC CSH C<sub>18</sub> Column and methanol scored highest, indicating the separation had the highest number of peaks with resolution  $\geq 2.0$  and a tailing  $\leq 1.5$ .

## Optimization

Although we were closer to the method development goal, the results from screening did not fully meet the criteria for success. We continued through the optimization step to improve the separation. During optimization we investigated the impact of gradient slope, column temperature, and pH. After each step we applied our scoring report to select the best conditions.

The first parameter we investigated was gradient slope by varying the gradient end point using the same gradient time. After applying our report we found that a gradient slope from 5-60% over 5 minutes provided the

best separation (Figure 4). With a goal of meeting the criteria for resolution between all the peaks, we then optimized column temperature using the same system setup. Our results indicated that 45 °C yielded the greatest resolution of all components and met all of the goals we set at the start of the development process, Figure 5.


|  <b>SCORING REPORT</b><br>Sample Set ID: 5181      Run Time: 7.0 Minutes<br>Result Set ID: 7285      Injection Volume: 1.00 ul<br>Processed Channel Descr.: PDA 270.0 nm (200-400)nm |         |                |        |             |                      |                           |           |        |                 |  |
|---|---------|----------------|--------|-------------|----------------------|---------------------------|-----------|--------|-----------------|--|
| Sample  | Column  | Strong Solvent | pH     | Total Peaks | Total Peaks Rs >=2.0 | Total Peaks Tailing <=1.5 | Lowest Rs | Min k* | RT of Last Peak |  |
| 1 Metoclopramide Rel Sub, 60%D  | CSH C18 | MeOH           | Low pH | 9           | 7                    | 9                         | 1.727     | 3.56   | 4.01            |  |
| 2 Metoclopramide Rel Sub, 70%D  | CSH C18 | MeOH           | Low pH | 9           | 7                    | 8                         | 1.592     | 3.43   | 3.63            |  |
| 3 Metoclopramide Rel Sub, 80%D  | CSH C18 | MeOH           | Low pH | 9           | 7                    | 7                         | 1.463     | 3.31   | 3.34            |  |
| 4 Metoclopramide Rel Sub, 90%D  | CSH C18 | MeOH           | Low pH | 9           | 7                    | 7                         | 1.346     | 3.21   | 3.11            |  |

Figure 4. Gradient slope optimization. Different gradient slopes were explored by decreasing the % of organic at the end of the gradient from 5-90% to 80, 70, and 60% over 5 minutes. A gradient with 5-60% of methanol over 5 minutes had the highest score, indicating best separation with highest number of peaks with resolution  $\geq 2.0$  and a tailing  $\leq 1.5$ .


|  <b>SCORING REPORT</b><br>Sample Set ID: 5325, 5232      Run Time: 7.0 Minutes<br>Result Set ID: 7236, 7205      Injection Volume: 1.00 ul<br>Processed Channel Descr.: PDA 270.0 nm (200-400)nm |         |                |        |             |                      |                           |        |           |                 |  |
|---|---------|----------------|--------|-------------|----------------------|---------------------------|--------|-----------|-----------------|--|
| Sample  | Column  | Strong Solvent | pH     | Total Peaks | Total Peaks Rs >=2.0 | Total Peaks Tailing <=1.5 | Min k* | Lowest Rs | RT of Last Peak |  |
| 1 Metoclopramide Rel Sub, 45C   | CSH C18 | MeOH           | Low pH | 9           | 8                    | 9                         | 3.27   | 2.280     | 3.87            |  |
| 2 Metoclopramide Rel Sub, 50C   | CSH C18 | MeOH           | Low pH | 9           | 7                    | 9                         | 3.04   | 1.816     | 3.79            |  |
| 3 Metoclopramide Rel Sub, 40C   | CSH C18 | MeOH           | Low pH | 9           | 7                    | 9                         | 3.54   | 1.757     | 3.99            |  |

Figure 5. Column temperature optimization. The temperatures investigated included 40, 45, and 50 °C. Method at 45 °C scored highest with greatest number of peaks with a resolution of  $\geq 2.0$ , indicating best separation. At this stage, although we had met all our criteria, we also investigated impact of pH on the chromatographic separation. Often, small changes in pH can have a great impact on the retention of ionizable compounds. We performed separations at pH 2.15, 3.0, and 4.0 using the existing mobile phases defined in the protocol, Figure 6. For pH 3.0 and 4.0, we used Auto-Blend Plus Technology to blend formic acid and ammonium hydroxide solutions, methanol, and water already on the system to deliver mobile phases with constant pH. Our results showed large changes in selectivity as we moved to the higher pH and that, ultimately, pH 2.15 yielded the best separation, Figure 7.

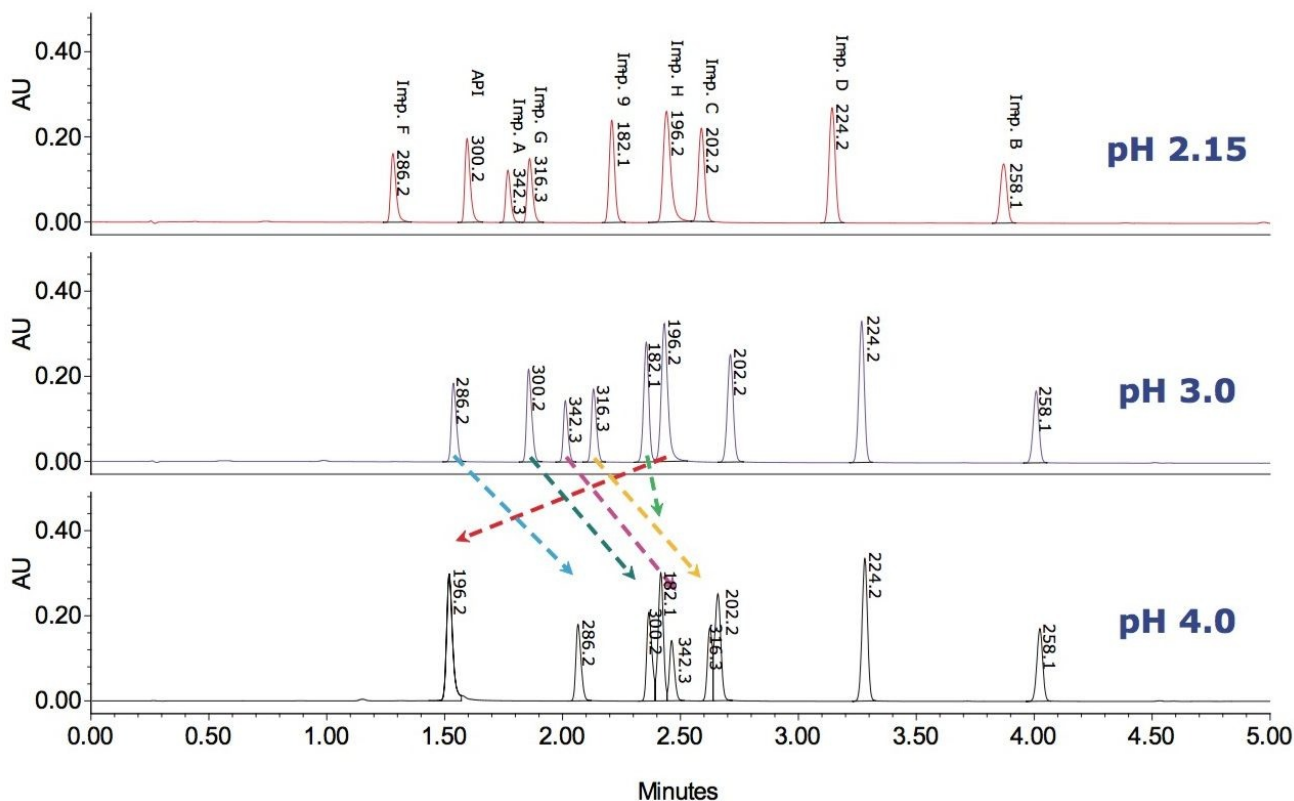


Figure 6. pH optimization to study the impact of pH on the separation of metoclopramide and related compounds. Peaks were tracked by mass detection using an ACQUITY QDa Detector. The best separation conditions were found to be at a pH of 2.15.

| Empower 3 SOFTWARE               |         | SCORING REPORT            |          | Sample Set ID: 5325, 6461                          |                      | Run Time: 7.5, 7.0 Minutes |        |           |                 |
|----------------------------------|---------|---------------------------|----------|--|----------------------|----------------------------|--------|-----------|-----------------|
|                                  |         | Result Set ID: 7263, 7273 |          | Processed Channel Descr.: PDA 270.0 nm (200-400)nm |                      | Injection Volume: 1.00 ul  |        |           |                 |
| Sample                           | Column  | Strong Solvent            | pH       | Total Peaks  | Total Peaks Rs >=2.0 | Total Peaks Tailing <=1.5  | Min k* | Lowest Rs | RT of Last Peak |
| 1 Metoclopramide Rel Sub         | CSH C18 | MeOH                      | Low pH   | 9  | 8                    | 9                          | 3.27   | 2.280     | 3.87            |
| 2 Metoclopramide Rel Sub, pH 3.0 | CSH C18 | MeOH                      | pH = 3.0 | 9  | 7                    | 9                          | 4.12   | 1.687     | 4.01            |
| 3 Metoclopramide Rel Sub, pH 4.0 | CSH C18 | MeOH                      | pH = 4.0 | 9  | 5                    | 4                          | 4.06   | 0.632     | 4.02            |

Figure 7. pH optimization. The method with a mobile phase pH of 2.15 scored highest, indicating best separation conditions.

## Final UPLC method conditions

LC System: ACQUITY UPLC H-Class

Column: ACQUITY UPLC CSH C18, 1.7- $\mu$ m, 2.1 x 50

|                    |   |
|--------------------|---|
|                    | mm                                      |
| Column temp.:      | 45 °C                                   |
| Injection volume:  | 1.0 µL                                  |
| Flow rate:         | 0.6 mL/min                              |
| Mobile phase A:    | 125 mM Formic acid in water             |
| Mobile phase C:    | Water                                   |
| Mobile phase D2:   | Methanol                                |
| Wash solvents:     | Purge/Sample wash: 50:50 water/methanol |
| Seal wash:         | 90:10 water/acetonitrile                |
| PDA detector:      | ACQUITY UPLC PDA                        |
| PDA settings:      | 210-400 nm (derived at 270 nm)          |
| MS detector:       | ACQUITY QDa (Extended Performance)      |
| Scan mode:         | 100-400 <i>m/z</i>                      |
| Ionization mode:   | ESI+, ESI-                              |
| Probe temp.:       | 600 °C                                  |
| Sampling rate:     | 10 pts/sec                              |
| Capillary voltage: | 0.8 kV (pos/neg)                        |
| Cone voltage:      | 15 V                                    |

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Data: Centroid

System control, data acquisition, and analysis: Empower 3 FR2 CDS Software


## Gradient

| Step | Time<br>(min) | Solvent<br>A % | Solvent<br>C % | Solvent<br>D2 (%) |
|------|---------------|----------------|----------------|-------------------|
| 1.0  | Initial       | 10.0           | 85.0           | 5.0               |
| 2.0  | 5.0           | 10.0           | 30.0           | 60.0              |
| 3.0  | 5.5           | 10.0           | 30.0           | 60.0              |
| 4.0  | 5.6           | 10.0           | 85.0           | 5.0               |
| 5.0  | 7.0           | 10.0           | 85.0           | 5.0               |

## Final UPLC method

To verify performance of the developed UPLC method, we evaluated repeatability of replicate injections of the sample. The system suitability of five replicate injections was determined according to specifications defined in the USP General Chapter, <621> Chromatography.<sup>2</sup> Results of the method system suitability for each component are shown in Table 2.

The retention times and area repeatability were well below the USP specification of less than 2.0% RSD. The USP resolution between all the peaks was  $\geq 2.5$ , which is above the general USP requirements of  $\geq 1.5$ . The system suitability results of replicate injections were excellent. Further validation testing can be done automatically using Empower Method Validation Manager (MVM) Software.

|   |                                       |
|---|---------------------------------------|
|  | Report Method: System Suit_Sum Report |
|   | Sample Set ID: Sample Set Id 2622     |
|   | Result Set ID: Result Set Id 2660     |
|   | Channel Name: PDA 270                 |

|   | Name   | # of Inj. | %RSD RT | %RSD Peak Areas | Ave USP Resolution | Ave USP Tailing |
|---|--------|-----------|---------|-----------------|--------------------|-----------------|
| 1 | Imp. F | 5         | 0.07    | 0.19            |                    | 1.2             |
| 2 | API    | 5         | 0.06    | 0.22            | 6.7                | 1.3             |
| 3 | Imp. A | 5         | 0.06    | 0.21            | 3.4                | 1.2             |
| 4 | Imp. G | 5         | 0.07    | 0.23            | 2.5                | 1.2             |
| 5 | Imp. 9 | 5         | 0.06    | 0.19            | 9.2                | 1.1             |
| 6 | Imp. H | 5         | 0.06    | 0.19            | 4.2                | 1.4             |
| 7 | Imp. C | 5         | 0.06    | 0.31            | 2.5                | 1.1             |
| 8 | Imp. D | 5         | 0.05    | 0.21            | 9.0                | 1.1             |
| 9 | Imp. B | 5         | 0.04    | 0.21            | 13.0               | 1.1             |

Table 2. System suitability results for five replicate sample injections acquired using an ACQUITY UPLC H-Class System.

## Conclusion

Following a systematic protocol, we have successfully developed a UPLC method for the separation of metoclopramide and related compounds. The criteria for success with a goal of separating all nine components, achieving a resolution of  $\geq 2.0$ , tailing of  $\leq 1.5$ , and retention factor ( $k^*$ )  $\geq 3.0$ , were met.

Using the ACQUITY QDa Detector in conjunction with UV detection and the ACQUITY UPLC H-Class System streamlined the method development process by removing the need for multiple chromatographic runs to confirm the identity of peaks by retention times.

Using a single injection, instead of nine individual sample injections, we were able to quickly identify components and track elution order of peaks during the method development study.

Finally, the use of ApexTrack in Empower Software enabled consistent evaluation of chromatograms for fair comparison across the development process. Empower custom calculations and reporting allowed us to generate a scoring report to easily identify the best conditions at each step in our protocol.

Overall, using a defined systematic protocol with the UPLC system, detectors, and its column chemistries

enables analytical laboratories to quickly and efficiently develop chromatographic methods. Methods developed in this manner are typically more reproducible, which allows laboratories to have a higher validation success rate.

## References

1. General Chapter, <1226>, Verification of Compedial Method, USP36-NF31, The United States Pharmacopeia Convention, official December 1, 2013.
2. USP General Chapter, <621>, Chromatography, USP36-NF31, The United States Pharmacopeia Convention, official December 1, 2013.
3. Berthelette KD, Summers M, Fountain KJ. Ensuring Data Quality by Benchmarking System Performance Using Waters Neutrals Quality Control Reference Material. Waters Corp. 2013; 720004622en.

## Featured Products

- [ACQUITY UPLC H-Class System <https://www.waters.com/10138533>](https://www.waters.com/10138533)
- [ACQUITY QDa Mass Detector <https://www.waters.com/134761404>](https://www.waters.com/134761404)
- [ACQUITY UPLC PDA Detector <https://www.waters.com/514225>](https://www.waters.com/514225)
- [Empower Chromatography Data Software <https://www.waters.com/513188>](https://www.waters.com/513188)

### Available for purchase online:

- [ACQUITY UPLC CSH C18, 1.7 µm, 2.1 mm X 50 mm Column <https://www.waters.com/waters/partDetail.htm?partNumber=186005296>](https://www.waters.com/waters/partDetail.htm?partNumber=186005296)
- [CORTECS UPLC C18+, 1.6 µm, 2.1 mm X 50 mm Column <https://www.waters.com/waters/partDetail.htm?partNumber=186007114>](https://www.waters.com/waters/partDetail.htm?partNumber=186007114)
- [ACQUITY UPLC CSH Phenyl-Hexyl, 1.7 µm, 2.1 mm X 50 mm Column <https://www.waters.com/waters/partDetail.htm?partNumber=186005406>](https://www.waters.com/waters/partDetail.htm?partNumber=186005406)
- [ACQUITY UPLC HSS PFP, 1.8 µm, 2.1 mm X 50 mm Column <https://www.waters.com/waters/partDetail.htm?partNumber=186005965>](https://www.waters.com/waters/partDetail.htm?partNumber=186005965)

· [LCMS QC Reference Standard <https://www.waters.com/waters/partDetail.htm?partNumber=186006963>](https://www.waters.com/waters/partDetail.htm?partNumber=186006963)

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