

アプリケーションノート

Robust and Rapid Method for Analysis of Active Pharmaceutical Ingredients in Multi-Component Cold and Flu Medication

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Abstract

A UPLC method was successfully developed for the analysis of active pharmaceutical ingredients in common over-the-counter cold and flu drug formulations. In this application note, we present the development of a MS-compatible UPLC method for the simultaneous determination of eight active pharmaceutical ingredients (APIs) found in common over-the-counter (OTC) cold and flu medication. These APIs include acetaminophen, dextromethorphan hydrobromide, phenylephrine hydrochloride, chlorpheniramine maleate, ibuprofen, pseudoephedrine hydrochloride, duaifenesin, and doxylamine succinate.

Benefits

- Fast and effective development of reproducible and robust methods with a systematic approach
- Quick and accurate identification of sample components using mass detection with the ACQUITY QDa Mass Detector
- Confirm spectral peak purity using the ACQUITY UPLC PDA and ACQUITY QDa detectors

Introduction

Pharmaceutical drug products used for treatment of common cold and flu often contain multiple active ingredients to target different symptoms and may include combination of decongestants, antihistamines, pain relievers, cough suppressants, and expectorants. These actives often exhibit different chemical characteristics such as a wide range of polarities, making chromatographic method development a challenging task.

Many of the methods reported in the literature for cold and flu medication are designed for analysis of individual components or for a particular dosage form. While other methods are suitable for simultaneous determination of multi-components in drug formulations, they utilize mobile phases with non-volatile buffers that are not compatible with mass detection.¹⁻³ Addition of mass detection to the pharmaceutical analysis workflows can often enable a quick and accurate identification of new or unknown sample components during the development or to confirm peak purity in routine assay testing.

In this application note, we present the development of a MS-compatible UPLC method for the simultaneous determination of eight active pharmaceutical ingredients (APIs) found in common over-the-counter (OTC) cold and flu medication. These APIs include acetaminophen, dextromethorphan hydrobromide, phenylephrine hydrochloride, chlorpheniramine maleate, ibuprofen, pseudoephedrine hydrochloride, duaifenesin, and doxylamine succinate. A systematic protocol that includes scouting, screening, and optimization steps is employed to ensure faster and more effective development of robust

and reproducible method. Results from each step are analyzed using custom calculations and custom reports of the Empower 3 Chromatographic Data Software. Both the UV and mass spectral data from the ACQUITY QDa Mass Detector is utilized for accurate identification and tracking all of the components during the development process. Finally, the developed method is used to analyze commercially available over-the-counter cold and flu medication.

Experimental

Standard preparations

Sample solution with APIs for method development

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 a standard diluent (90:10 water/methanol) to a final working concentration of 100 µg/mL of each analyte.

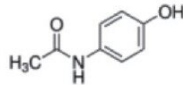
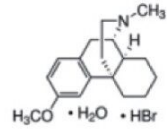
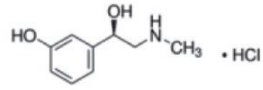
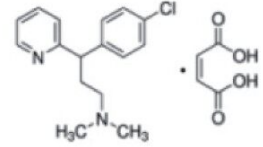
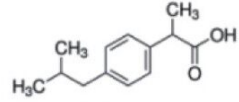
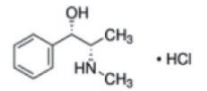
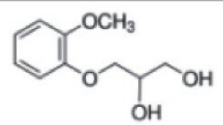
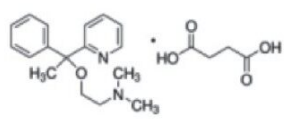
| Compound | Formula | Monoisotopic mass (m/z) | Structure |
|-------------------------------|---|-----------------------------|---|
| Acetaminophen | C ₈ H ₉ NO | 151.06 |  |
| Dextomethorphan hydrobromide | C ₁₈ H ₂₆ BrNO | 351.12 Free base: 271.19 |  |
| Phenylephrine hydrochloride | C ₉ H ₁₄ ClNO ₂ | 203.07 Free base: 167.09 |  |
| Chlorpheniramine maleate | C ₂₀ H ₂₃ ClN ₂ O ₄ | 390.13 Free base: 274.12 |  |
| Ibuprofen | C ₁₃ H ₁₈ O ₂ | 206.13 |  |
| Pseudoephedrine hydrochloride | C ₁₀ H ₁₆ O ₄ | 201.09 Free base: 165.11 |  |
| Guaifenesin | C ₁₀ H ₁₄ O ₄ | 198.09 |  |
| Doxylamine succinate | C ₂₁ H ₂₈ N ₂ O ₅ | 388.20 Free base: 270.17 |  |

Table 1. List of active pharmaceutical ingredients (APIs) for chromatographic method development.

Cold and flu drug formulations

Over-the-counter cold and flu medication tested in this study included syrup, tablets, and caplets. All samples were prepared and diluted to the working concentration with sample diluent containing 90:10 water/methanol as outlined in Table 2. Each working solution was then filtered through 0.2 μm GHP syringe filter prior to analysis.

| Formulation sample | API, mg per dose | Sample solution preparation (90:10 water/methanol diluent) | Working sample concentration (µg/mL) |
|---|--|---|--|
| Mucinex® Cold, Flu and Sore Throat Maximum Strength Syrup | Each 20 mL of syrup contains • Acetaminophen, 650 • Dextromethorphan HBr, 20 • Guaifenesin, 400 • Phenylephrine HCl, 10 | 20 mL of syrup diluted with sample diluent to the working concentration. | Acetaminophen, 325 Dextromethorphan HBr, 10 Guaifenesin, 200 Phenylephrine HCl, 5 |
| Vicks DayQuil™ Severe Caplets | Each caplet contains • Acetaminophen, 325 • Dextromethorphan HBr, 10 • Guaifenesin, 200 • Phenylephrine HCl, 5 | Caplets dissolved in diluent via sonication for 30 minutes and diluted to the working concentration. | Acetaminophen, 325 Dextromethorphan HBr, 10 Guaifenesin, 200 Phenylephrine HCl, 5 |
| Vicks NyQuil™ Severe Caplets | Each caplet contains • Acetaminophen, 325 • Dextromethorphan HBr, 10 • Phenylephrine HCl, 5 • Doxylamine succinate, 6.25 | | Acetaminophen, 325 Dextromethorphan HBr, 10 Phenylephrine HCl, 5 Doxylamine succinate, 6.25 |
| CVS Sinus PE + Allergy Tablets | Each tablet contains • Chlorperinamine maleate, 4 • Phenylephrine HCl, 10 | Tablets dissolved in diluent via sonication for 30 minutes. Diluted to volume to make working solution. | Chlorperinamine maleate, 80 Phenylephrine HCl, 200 |
| TYLENOL® Cold & Flu Severe Caplets | Each caplet contains • Acetaminophen, 325 • Dextromethorphan HBr, 10 • Guaifenesin, 200 • Phenylephrine HCl, 5 | Caplets dissolved in diluent via sonication for 30 minutes and diluted to the working concentration. | Acetaminophen, 325 Dextromethorphan HBr, 10 Guaifenesin, 200 Phenylephrine HCl, 5 |

Table 2. Over-the-counter cold and flu drug formulations with respective preparation scheme.

Method development conditions

LC system:

ACQUITY UPLC H-Class PLUS with Column Manager (Active) and Solvent Select Valve

Columns:

All columns with dimension of 2.1 × 50 mm

ACQUITY UPLC CSH C₁₈, 1.7 µm

ACQUITY UPLC CORTECS™ T3, 1.6 µm

ACQUITY UPLC CORTECS Phenyl, 1.6 µm

ACQUITY UPLC HSS PFP, 1.8 µm

ACQUITY UPLC BEH C₁₈, 1.7 µm

ACQUITY UPLC BEH Shield RP18, 1.7 µm

Column temp.:

40 °C

| | |
|----------------------------|--|
| Injection volume: | 1.0 µL |
| Flow rate: | 0.6 mL/min |
| Mobile phase A: | 125 mM Formic acid in water |
| Mobile phase B: | 125 mM Ammonium hydroxide in water |
| Mobile phase C: | Water |
| Mobile phase D1: | Acetonitrile |
| Mobile phase D2: | Methanol |
| Separation: | Gradient with 5–90% organic solvent over 5 minutes |
| Purge/sample wash solvent: | 70:30 water/methanol |
| Seal wash: | 90:10 water/acetonitrile |
| UV detector: | ACQUITY UPLC PDA 200–500 nm (Derived at 220 nm) |

Final method conditions

| | |
|-------------------|---|
| Column: | ACQUITY UPLC BEH C ₁₈ , 2.1 × 50 mm, 1.7 µm |
| Column temp.: | 40 °C |
| Injection volume: | 1.0 µL |
| Flow rate: | 0.6 mL/min |
| Mobile phase A: | 10 mM Ammonium acetate in water with 0.2% of ammonium hydroxide |
| Mobile phase B: | Methanol with 0.2% ammonium hydroxide |

Purge/sample wash solvent: 70:30 water/methanol

Seal wash: 90:10 water/acetonitrile

PDA settings: 200–500 nm (derived at 215 nm)

Gradient:

| Step | Time (min) | Solvent A (%) | Solvent B (%) |
|------|------------|---------------|---------------|
| 1 | Initial | 95.0 | 5.0 |
| 2 | 5.0 | 10.0 | 90.0 |
| 3 | 5.5 | 10.0 | 90.0 |
| 4 | 5.6 | 95.0 | 5.0 |
| 5 | 7.5 | 95.0 | 5.0 |

MS conditions (development and final method)

MS detector: ACQUITY QDa (Extended Performance)

Ionization mode: ESI+, ESI-

Acquisition range: 100–400 *m/z*

Capillary voltage: 0.8 kV (pos/neg)

Cone voltage: 5 V

Probe temp.: 600 °C

Data: Centroid

Results and Discussion

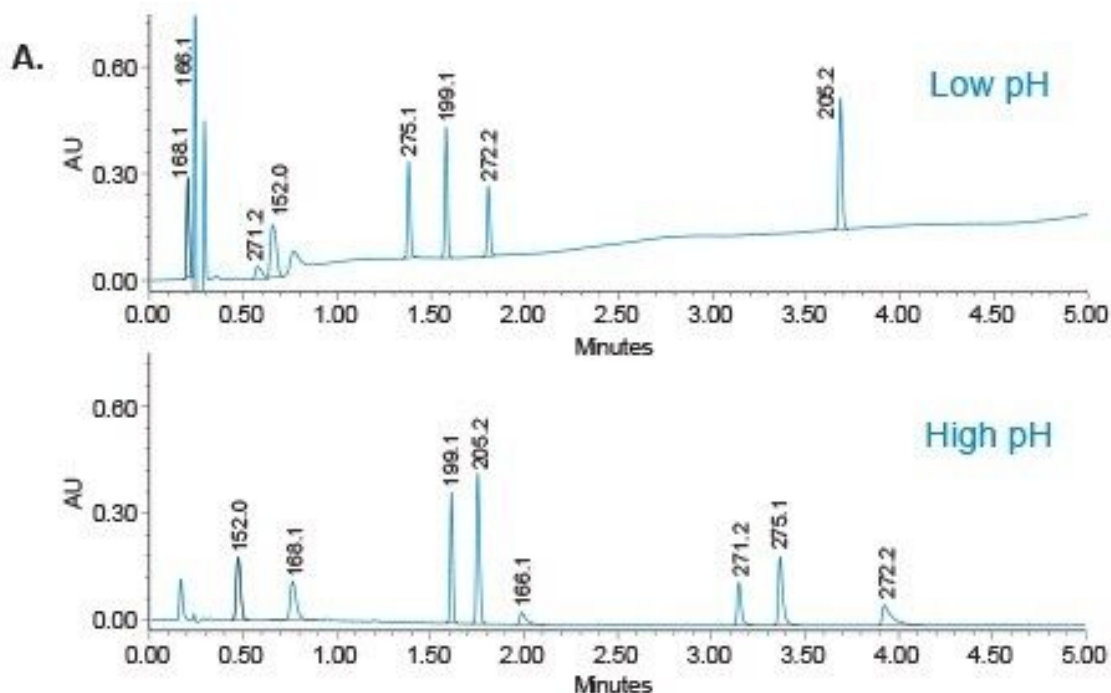
Method development

A UPLC method for analysis of APIs found in common over-the-counter (OTC) cold and flu medication was developed following a systematic protocol.⁴ A systematic protocol is based on a consistent evaluation of major selectivity factors, which enables development of robust and reliable methods.

Before beginning the study, we defined the separation goals and selected a chromatographic system. The goal of our study was to separate all APIs with minimum resolution of ≥ 2.0 between the peaks, peak tailing of ≤ 1.5 , and retention factor (k^*) ≥ 2.0 . We used the ACQUITY UPLC H-Class PLUS System configured with a Column Manager and Solvent Select Valve to maximize flexibility of the method development. The ACQUITY QDa Mass Detector in combination with the ACQUITY PDA Detector allowed quick identification of all components and possible co-elution during the development process.

After defining the separation goals and system, we started the study following a three step approach that included rapid scouting, screening, and optimization.

The goal of the rapid scouting was to quickly identify separation condition that provided the best retention of our analytes, as well as to determine the best separation mode (reverse-phase or HILIC). The low and high pH experiments were performed on an ACQUITY UPLC CSH C₁₈ Column using stock solutions of 125 mM formic acid and 125 mM ammonium hydroxide with a gradient of 5–90% of acetonitrile solvent over five minutes. The chromatographic data showed that the retention of our analytes changed under low and high pH conditions (Figure 1). The mass data of the ACQUITY QDa was used to identify each peak by mass-to-charge (m/z) ratio (Figure 1A). The MS Peak Tracking feature and report of the Empower 3 Software enabled us to monitor elution order of each analyte over the pH experiments (Figure 1B). The report table displayed the retention time for each peak with a specific m/z over the chromatographic runs.



B.

Empower 3

MS Peak Tracking Report

Sample Set ID: 3450 Processed Channel: 220.0nm

Retention Time Summarized by Assigned Mass

| SampleName | 152.0 (min) | 166.1 (min) | 168.1 (min) | 199.1 (min) | 205.2 (min) | 271.2 (min) | 272.2 (min) | 275.1 (min) |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1 APIs, Low pH | 0.658 | 0.243 | 0.204 | 1.581 | 3.583 | 0.576 | 1.806 | 1.381 |
| 2 APIs, High pH | 0.474 | 1.988 | 0.765 | 1.613 | 1.753 | 3.148 | 3.924 | 3.367 |

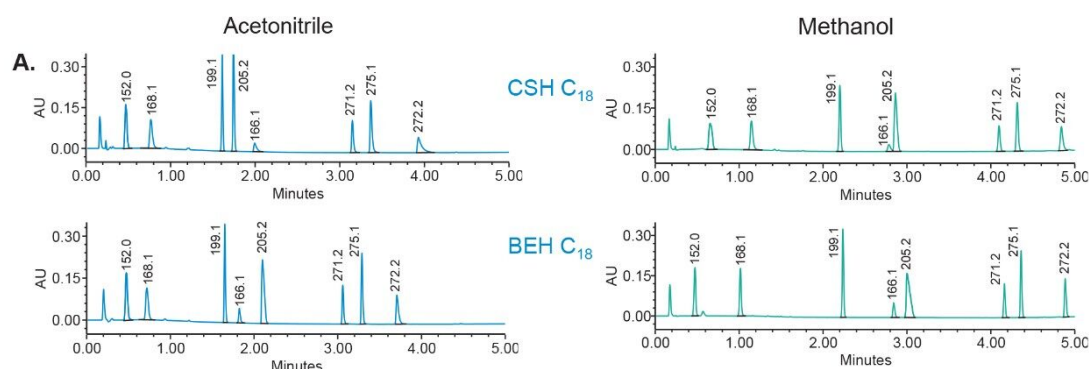
Figure 1. Rapid scouting. Chromatographic separation with mass-to-charge (m/z) ratio for each peak (A). Empower MS Peak Tracking report table displays retention time of each peak (B).

Custom calculations and custom scoring report of the Empower 3 Software were used to quickly identify which condition provided best separation (Figure 2). The low and high pH separations were scored for best conditions by identifying number of peaks that met the performance goals. In this case, the high pH provided best retentivity for all analytes, hence was chosen for the next step of the method development.

| Empower ³ | | SCORING REPORT | | | | | Run Time: 7.5 Minutes | | | | |
|----------------------|---------|---------------------|----------------|-------------|----------------------------|--------------------------------|-----------------------|-----------|---------------------------|-----------------|--|
| | | Sample Set ID: 3450 | | | Processed Channel: 220.0nm | | | | Injection Volume: 1.00 ul | | |
| | pH | Column | Strong Solvent | Total Peaks | Total Peaks Rs \geq 2.0 | Total Peaks Tailing \leq 1.5 | Min k ² | Lowest Rs | RT of First Peak | RT of Last Peak | |
| 1 | High pH | CSH C18 | ACN | 8 | 7 | 4 | 0.98 | 4.817 | 0.474 | 3.92 | |
| 2 | Low pH | CSH C18 | ACN | 8 | 5 | 8 | -0.15 | 1.355 | 0.204 | 3.68 | |

Figure 2. Empower 3 scoring report for rapid scouting. Conditions with best separation ranked highest based on the numbers of peaks that met the performance goals.

The high pH condition from the scouting step was screened with an ACQUITY UPLC CSH C₁₈ and ACQUITY UPLC BEH C₁₈ columns using methanol and acetonitrile solvents, respectively (Figure 3). The separation was performed using the same gradient as in the rapid scouting step. The chromatographic data showed that each condition provided an acceptable separation between components (Figure 3A). The scoring report was used to analyze the chromatographic data and showed that the ACQUITY UPLC BEH C₁₈ with methanol provided best separation with highest number of peaks with the USP resolution \geq 2.0 and a tailing \leq 1.5 (Figure 3B). Using this condition, we moved forward to the optimization phase.



B.

| Empower ³ | | SCORING REPORT | | | | | Run Time: 7.5 Minutes | | | | |
|----------------------|----------|---------------------------|------|-------------|------------------------------------|--------------------------------|-----------------------|------------------|---------------------------|-----------------|------|
| | | Sample Set ID: 3485, 4097 | | | Processed Channel: 220 nm, 220.0nm | | | | Injection Volume: 1.00 ul | | |
| Sample | Column | Strong Solvent | pH | Total Peaks | Total Peaks Rs \geq 2.0 | Total Peaks Tailing \leq 1.5 | Lowest Rs | Max Peak Tailing | Min k ² | RT of Last Peak | |
| 1 | APIs mix | BEH C18 | MeOH | High pH | 8 | 7 | 7 | 2.353 | 2.3 | 1.38 | 4.89 |
| 2 | APIs mix | CSH C18 | ACN | High pH | 8 | 7 | 5 | 4.562 | 3.3 | 1.36 | 3.93 |
| 3 | APIs mix | BEH C18 | ACN | High pH | 8 | 7 | 5 | 4.675 | 2.0 | 1.38 | 3.71 |
| 4 | APIs mix | CSH C18 | MeOH | High pH | 8 | 6 | 7 | 1.212 | 1.5 | 2.27 | 4.84 |

Figure 3. Screening with columns and solvents. Chromatographic data (A). Empower 3 scoring report (B) shows that the ACQUITY UPLC BEH C₁₈ Column and methanol provided best separation.

Next, we optimized different chromatographic parameters including gradient slope, column temperature, pH, and wavelength. In addition, we investigated addition of MS-compatible buffers to the mobile phase to further improve separation and peak tailing for our analytes. It was found that addition of ammonium acetate to the mobile phase with 0.2% ammonium hydroxide improved chromatographic separation and reduced peak tailing (Figure 4).

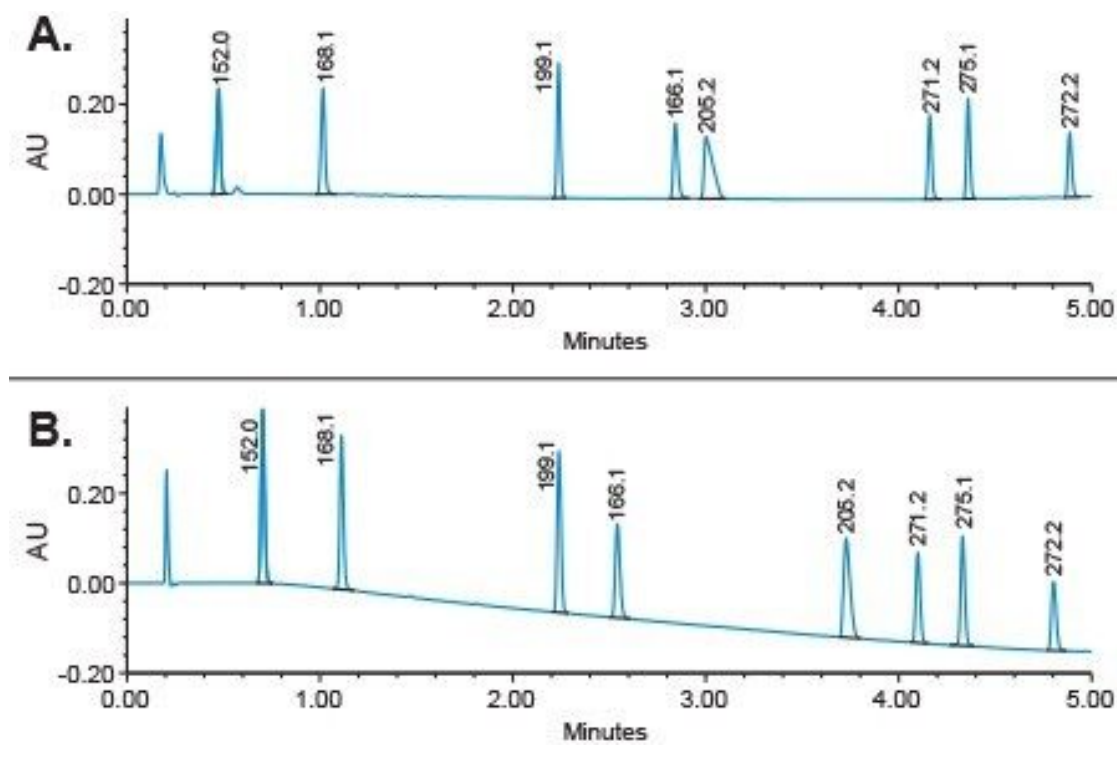


Figure 4. Mobile phase optimization. 0.1% ammonium hydroxide in water and methanol (A). 0.2% ammonium hydroxide in 10 mM ammonium acetate and in methanol (B). UV at 215 nm.

Final method

Performance of the developed UPLC method was measured by evaluating repeatability of five replicate injections of the sample according to the specifications defined in the USP General Chapter <621> Chromatography.⁵ The system suitability results (Figure 5) showed excellent repeatability of the retention times and peak areas with peak tailing ≤ 1.4 .

| | | |
|-----------|---|--|
| Empower 3 | System Suitability Report | |
| | Sample Set ID: 10781 Processed Channel: 215 nm | Run Time: 8.0 Minutes Injection Volume: 1.00 ul |

| Peak Results | | | | | | | | | |
|--------------|------------------|------------|-----------|--------|--------|---------|-----------------|--------------------|-----------------|
| | Name | Peak Label | # of Inj. | Ave RT | Ave k' | %RSD RT | %RSD Peak Areas | Ave USP Resolution | Ave USP Tailing |
| 1 | Acetaminophen | ACE | 5 | 0.706 | 2.2 | 0.21 | 0.35 | | 1.1 |
| 2 | Phenylephrine | PHE | 5 | 1.114 | 4.1 | 0.11 | 0.10 | 11.9 | 1.2 |
| 3 | Guaifenesin | GUA | 5 | 2.241 | 9.2 | 0.04 | 0.33 | 31.7 | 1.1 |
| 4 | Pseudoephedrine | PSE | 5 | 2.543 | 10.6 | 0.02 | 0.35 | 7.4 | 1.2 |
| 5 | Ibuprofen | IBU | 5 | 3.731 | 15.9 | 0.06 | 0.33 | 21.3 | 1.4 |
| 6 | Doxylamine | DOX | 5 | 4.100 | 17.6 | 0.01 | 0.29 | 6.8 | 1.2 |
| 7 | Chlorpheniramine | CHL | 5 | 4.332 | 18.7 | 0.01 | 0.23 | 5.7 | 1.1 |
| 8 | Dextromethorphan | DEX | 5 | 4.804 | 20.8 | 0.01 | 0.33 | 11.0 | 1.2 |

Figure 5. System suitability results of five replicate injections of the APIs sample mixture at 100 µg/mL.

In addition, reproducibility of the final method was investigated across three column batches using the ACQUITY UPLC BEH C₁₈ method validation kit (MVK). The ACQUITY UPLC method validation kits provide three batches of chromatographic media to judge the quality, reliability, and consistency of an analytical method. Reproducibility of the chromatographic separation across the three batches of the ACQUITY UPLC BEH C₁₈ Column was excellent (Figure 6).

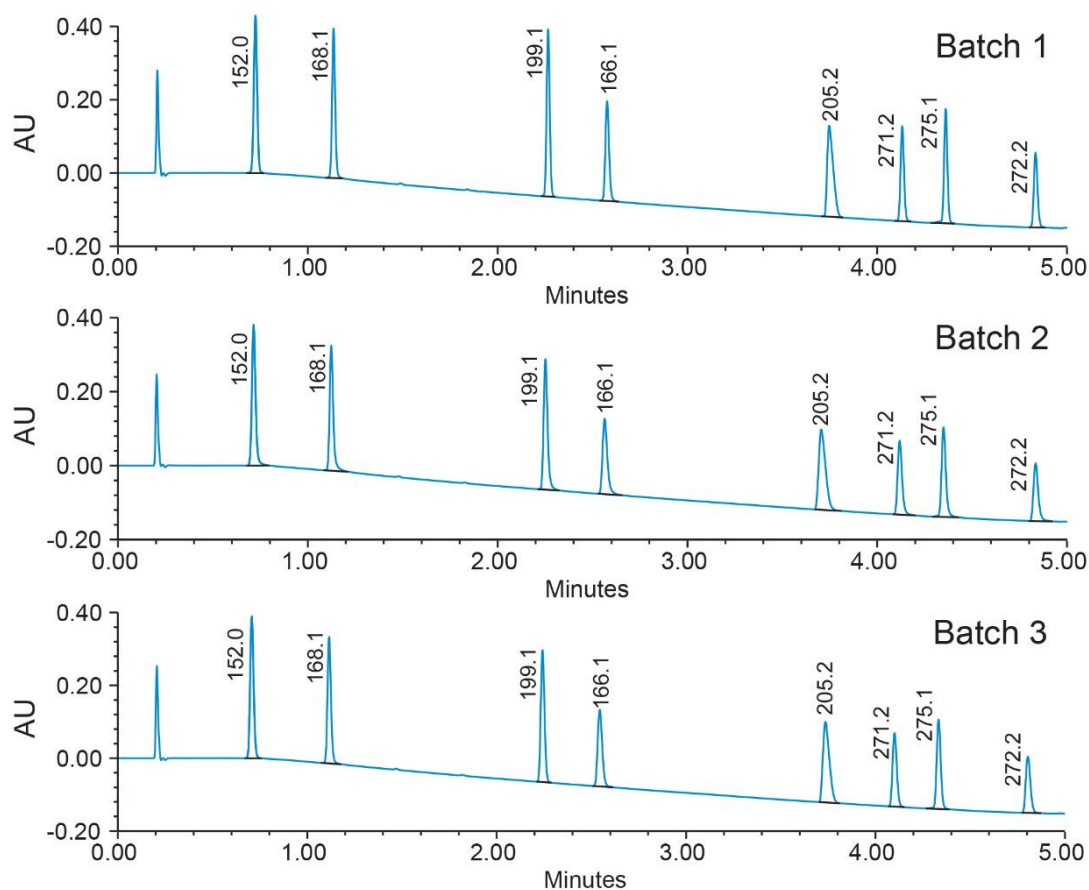


Figure 6. Chromatographic separation across three column batches of the ACQUITY UPLC BEH C₁₈ Column.

Analysis of cold and flu drug formulations

The over-the-counter cold and flu samples were analyzed to show that the developed method can separate active components from the formulation excipients. This is consistent with method specificity and often done by verifying peak purity or spectral homogeneity of the chromatographic peak.

In our study, we used UV in combination with MS spectral data to demonstrate that active components in cold and flu formulations were spectrally homogenous. As for the example, we are showing peak homogeneity determination of phenylephrine peak in Mucinex syrup sample (Figure 7). The UV peak purity plot showed that the peak purity angle was below the threshold angle, indicating that the phenylephrine was spectrally homogenous (Figure 7B). The Empower 3 Mass Analysis Window enabled us to look at the peak purity spectrum across each peak; that is at the leading, apex and trailing edge of the peak (Figure 7C). The top plot represents UV spectrum and the bottom MS spectrum, respectively. The MS spectrum showed presence of one mass (m/z) across the entire peak, specific for phenylephrine. Overall, both the UV

peak purity plot and mass spectral data confirmed that the phenylephrine was not coeluting with the components of the sample formulation.

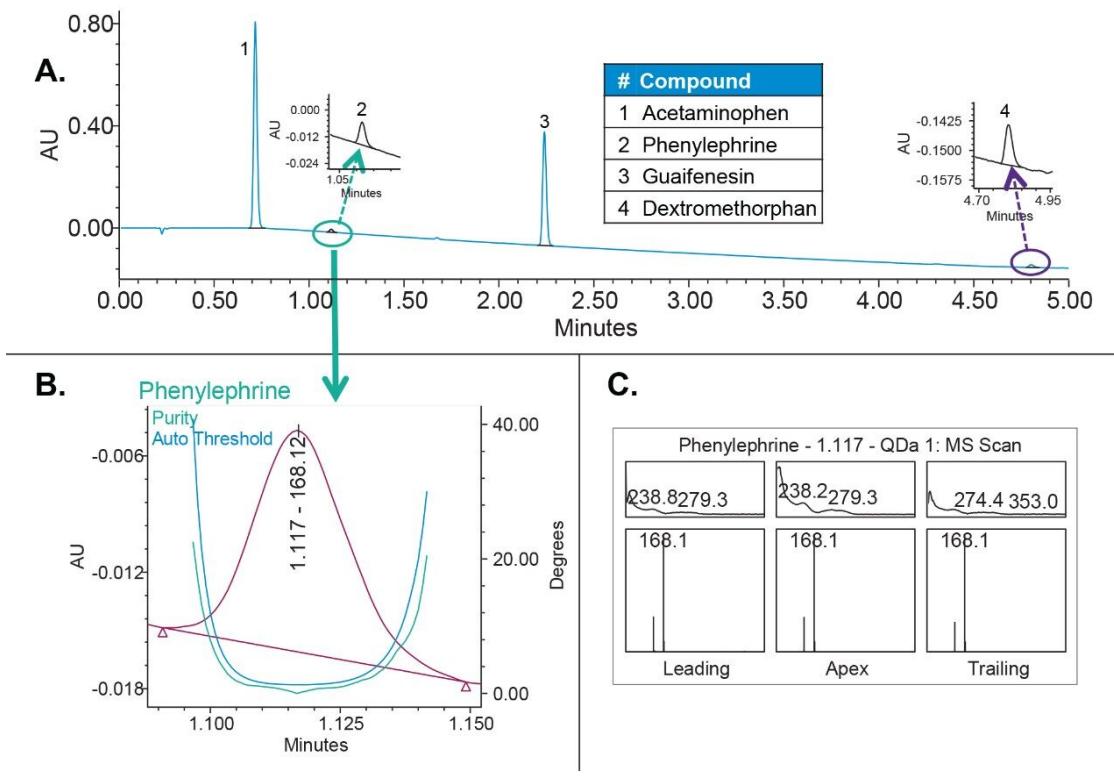


Figure 7. Mucinex syrup analysis with UV at 215 nm. Chromatographic separation (A). UV peak purity plot of the phenylephrine API (B). Empower 3 Mass Analysis window with peak purity spectrum for phenylephrine at the leading, apex, and trailing of the peak (C).

Overall, the analysis of all cold and flu drug commercially available medication showed that the active components were not subject to the interference with excipients of syrup, tablets, and caplets formulations (Figure 8). The Empower peak table for each medication showed that the purity angle was below the threshold angle, confirming that each active is spectrally homogenous or not coeluting with other components of the sample formulation.

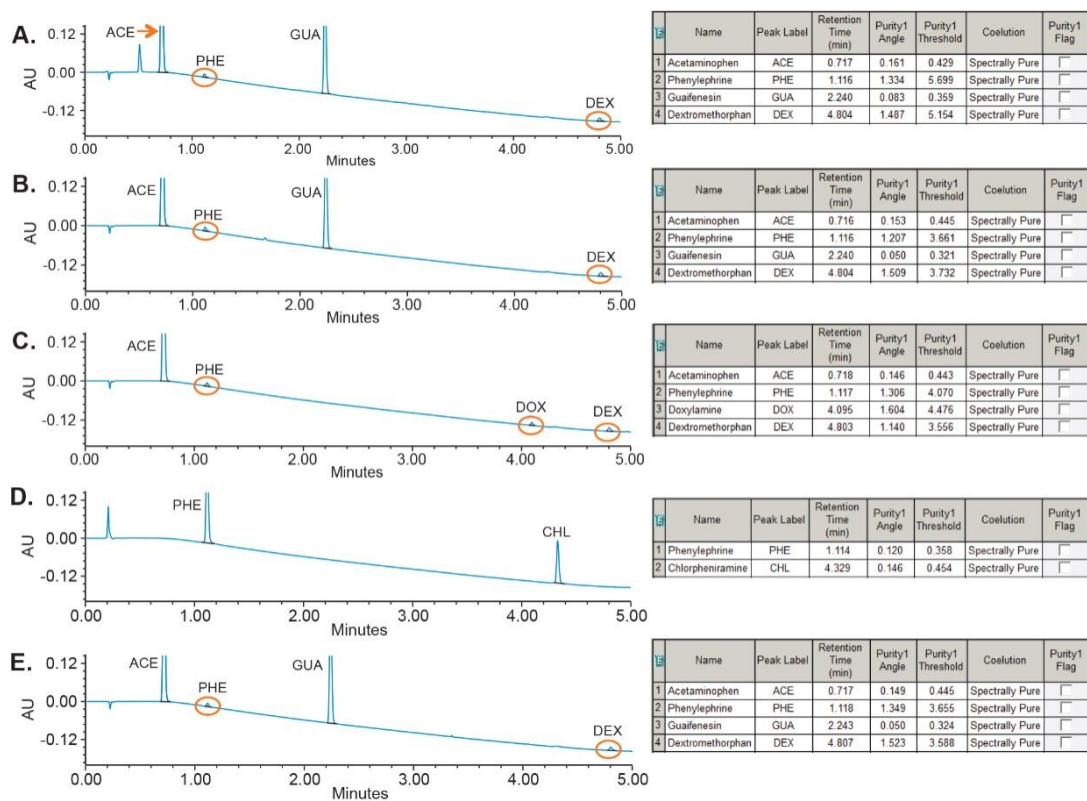


Figure 8. Over-the-counter cold and flu drug formulations and peak homogeneity evaluation. UV at 215 nm.

A. Mucinex Cold, Flu, and Sore Throat Maximum Strength Syrup

B. Vicks DayQuil Severe Caplets

C. Vicks NyQuil Severe Caplets

D. CVS Sinus PE + Allergy Tablets

E. TYLENOL Cold + Flu Severe Caplets

Conclusion

A UPLC method was successfully developed for the analysis of active pharmaceutical ingredients in common over-the-counter cold and flu drug formulations.

The ACQUITY UPLC H-Class PLUS with Column Manager and Solvent Select Valve streamlined method development by allowing us to screen multiple columns with different mobile phase in one chromatographic run. The ACQUITY QDa in conjunction with UV detection enabled quick identification of

sample components and monitoring elution order of peaks during the study. This eliminated the need to run multiple injections to identify peaks by retention times.

The MS peak tracking in Empower Software simplified method development by accurately tracking retention times of each component in sample by mass detection. Empower custom calculations and custom scoring report facilitated quick selection of best conditions at each step of the method development. The Empower Software peak purity determination confirmed spectral homogeneity of each active component in the pharmaceutical formulations.

Overall, combining ACQUITY UPLC PLUS System with UV and mass detection enables analytical laboratories to quickly and efficiently develop chromatographic methods.

References

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720006523, March 2019

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