# STREAMLINED METHOD DEVELOPMENT FOR ACTIVE PHARMACEUTICAL INGREDIENTS IN COLD AND FLU MEDICATION USING A SYSTEMATIC PROTOCOL

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## INTRODUCTION

**In this work, we present the development of a MS-compatible UPLC method for analysis of eightactive pharmaceutical ingredients (APIs) foundin common over-the-counter (OTC) cold and flumedication1.**

| Active Pharmaceutical Ingredient (API) | Formula | Monoisotopic mass (m/z) |
|---|---|---|
| Acetaminophen | $C_8H_9NO$ | 151.06 |
| Dextromethorphan HBr | $C_{18}H_{26}BrNO$ | 351.11 |
| Phenylephrine HCl | $C_9H_{14}ClNO_2$ | 203.07 |
| Chlorpheniramine maleate | $C_{20}H_{23}ClN_2O_4$ | 390.13 |
| Ibuprofen | $C_{13}H_{18}O_2$ | 206.13 |
| Pseudoephedrine HCl | $C_{10}H_{16}ClNO$ | 201.09 |
| Guaifenesin | $C_{10}H_{14}O_4$ | 198.09 |
| Doxylamine succinate | $C_{21}H_{28}N_2O_5$ | 388.20 |

Table 1. Compounds for method development.

## METHODS

### Sample Preparation

Separate stock solutions were prepared in methanol at 1.0 mg/mL and subsequently diluted with 90:10 water/ methanol to make a mixture with 100 µg/mL of each analyte.

### UPLC Method

| | |
|---|---|
| **LC System** | ACQUITY UPLC® H-Class PLUS with PDA & ACQUITY QDa™ Detectors |
| **Column** | ACQUITY UPLC BEH C18, 2.1 x 50, 1.7-µm |
| **Mobile Phase** | A: 10 mM Ammonium acetate in water with 0.2% of ammonium hydroxide<br>B: Methanol with 0.2% ammonium hydroxide |
| **Flow Rate** | 0.6 mL/min |
| **Column Temp.** | 40 ° C |
| **Sample Temp.** | 15 ° C |

**Gradient**

| Step | Time (minutes) | 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 |

Table 2. UPLC conditions of the final method.

## RESULTS AND DISCUSSION

### Method development

A systematic protocol2 that includes scouting, screening, and optimization steps was employed to develop a UPLC method for analysis of eight APIs (Table 1).

In the rapid scouting, low and high pH experiments were performed to quickly identify condition for best retentivity of the active ingredients. Mass spectral data from an ACQUITY QDa Detector was used to track the elution order of each analyte over the pH experiments (Figure 1).

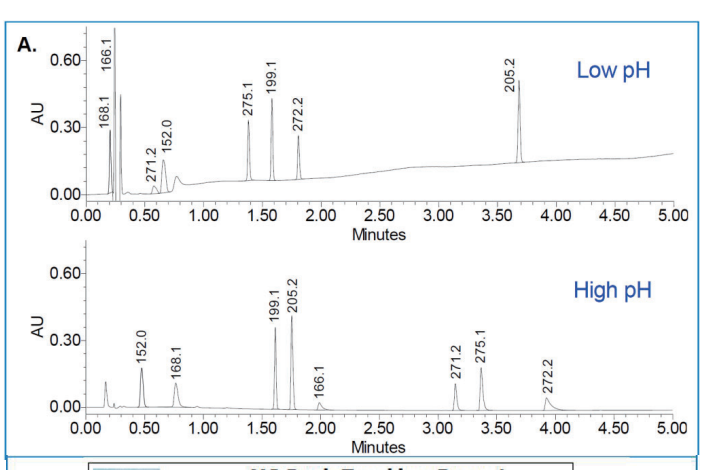



Figure 1. Rapid scouting with low and high pH. Separation with mass-to-charge (m/z) ratio for each peak (A). Empower MS Peak Tracking report displays retention time of each peak (B).

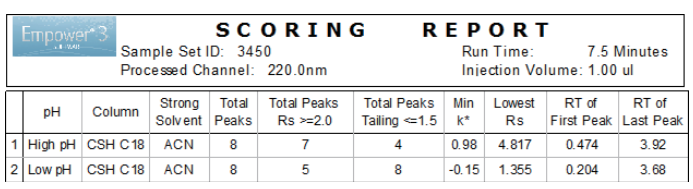



Figure 2. Empower 3 custom scoring report for rapid scouting. Conditions with best separation were ranked based on the numbers of peaks that met the performance goals. High pH ranked highest.

The high pH condition from the scouting step was screened with an ACQUITY UPLC CSH C18 and ACQUI- TY UPLC BEH C18 columns using methanol and acetonitrile solvents, respectively. The scoring report was used analyze the chromatographic data and showed that the ACQUITY UPLC BEH C18 with methanol provided best separation with the highest number of peaks for the USP resolution and peak tailing (Figure 3).

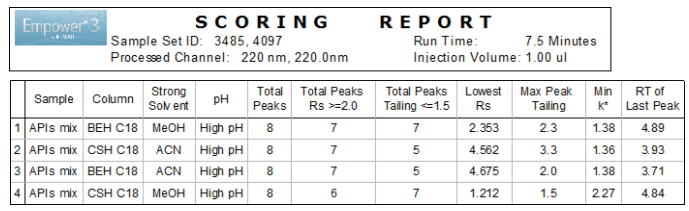



Figure 3. Screening with columns and solvents. Empower 3 scoring report shows that the ACQUITY UPLC BEH C18 column and methanol provided best separation..

The ACQUITY UPLC BEH C18 with methanol method was optimized by studying gradient slope, column temperature, pH and wavelength. In addition, the use of MS- compatible buffers was investigated to further improve separation and peak symmetry for the analytes. Addition of ammonium acetate to the mobile phase with 0.2% of ammonium hydroxide improved chromatographic separation and reduced peak tailing (Figure 4).

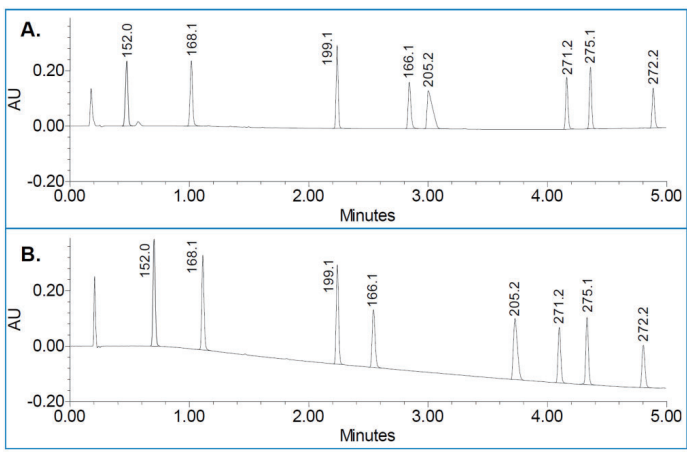
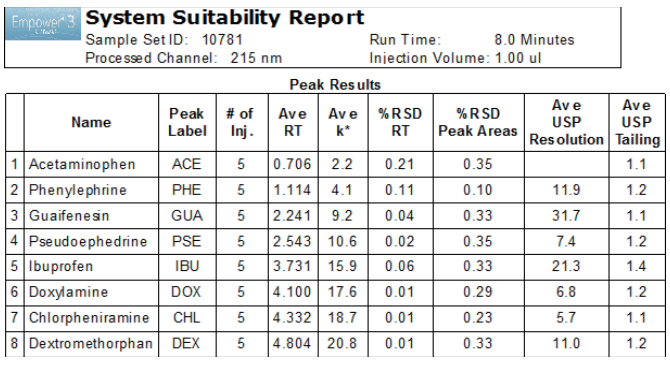



Figure 4. Mobile phase optimization. 0.1% ammonium hydroxide in water and methanol (A). 0.2% ammonium hydroxide in 10 mM ammonium acetate and in methanol (B). UV at 215 nm.

### System Suitability of the final method

Performance was measured by evaluating repeatability of five replicate injections of the sample according to the specifications defined in the USP General Chapter <621> Chromatography3. System suitability results were well below the USP acceptance criteria (Figure 5).




### Analysis of cold and flu medication

Over-the-counter (OTC) cold and flu samples were pre- pared in 90:10 water/methanol diluent to the working con- centration (Table 3) and filtered prior analysis

| Medication | API concentration in sample solution (µg/mL) |
|---|---|
| **Mucinex® Cold, Flu and Sore Throat Maximum Strength Syrup** | • Acetaminophen: 325<br>• Dextromethorphen HBr: 10<br>• Guaifenesin: 200<br>• Phenylephrine HCl: 5 |
| **VicksTM NyQuil Severe Caplets** | • Acetaminophen: 325<br>• Dextromethorphen HBr: 10<br>• Phenylephrine HCl: 5<br>• Doxylamine succinate: 6.25 |
| **CVS Health Sinus PE + Allergy tablets** | • Chlorpherinamine maleate: 80<br>• Phenylephrine HCl: 200 |
| **Tylenol® Cold & Flu Severe Caplets** | • Acetaminophen: 325<br>• Dextromethorphen HBr: 10<br>• Guaifenesin: 200<br>• Phenylephrine HCl: 5 |

Table 3. OTC cold and flu medication for analysis by UPLC

Spectral purity or homogeneity of each active ingredient was checked using peak purity tools in the Empower Software (Figure 6). The UV peak purity plot showed that the phenylephrine purity angle was below the threshold angle, confirming spectrally homogeneity (Figure 6B). The Empower 3 Mass Analysis Window showed one mass (m/z) across the entire peak, specific for phe- nylephrine (Figure 6C). Using both the UV and MS spec- tral data enabled spectral homogeneity confirmation to ensure that analytes are not subject to interference with any excipients of the sample formulations.

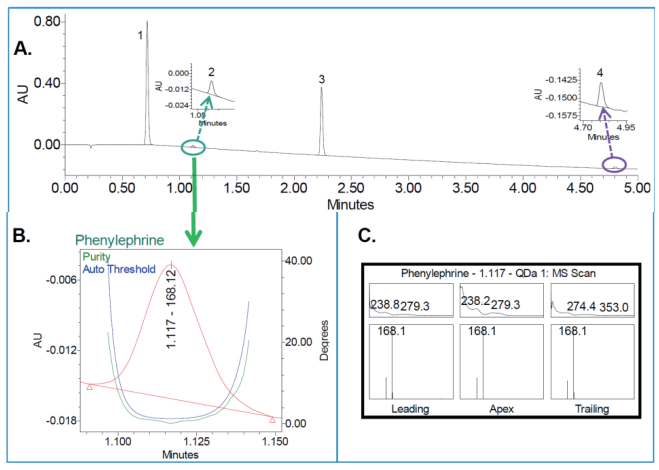



Figure 6. Peak purity determination of phenylephrine API. Mucinex syrup sample, UV at 215 nm (A). UV peak purity plot (B). Empower 3 Mass Analysis window with peak purity spectrum (C).

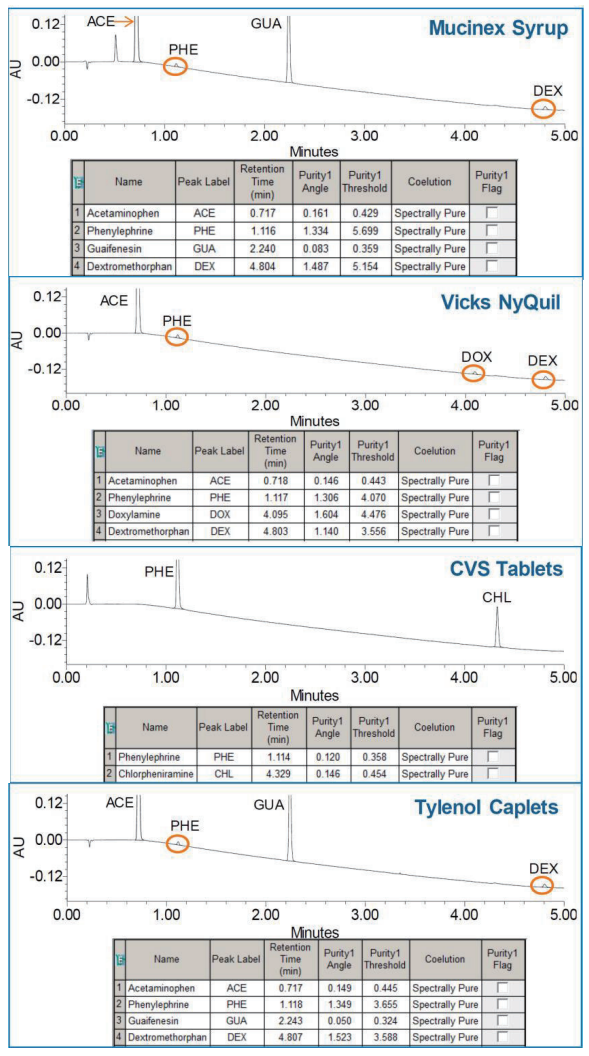



Figure 7. Analysis of cold and flu medication samples confirms that each API peak is spectrally homogenous

## CONCLUSION

**Quick and efficient development of reproducible and robust methods**

- Reduce development time with a systematic approach
- Automate columns and solvents screening using the UPLC H-Class PLUS System
- Easily identify and track peaks by using the ACQUITY QDa Mass Detector
- Perform quick data analysis and method selection with Empower scoring reports

**References**

1. Maziarz M, Rainville P. Robust and Rapid Method for Analysis of Active Pharmaceutical Ingredients in Multi-Component Cold and Flu Medication, Waters Application Note. 720006523EN. 2019.
2. Maziarz M, McCarthy SM, Wrona M. Improving Effectiveness in Method Development by Using a Systematic Screening Protocol for a USP MethodWaters Application Note 720005026EN. 2014
3. USP General Chapter, <621>, USP45-NF36, Chromatography, The United States Pharmacopeia Convention, Official August 2017

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