# Modernization of USP Monographs for Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic and Nasal Solutions

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## INTRODUCTION

**The United States Pharmacopeia (USP) is modernizing current *monographs* for Chemical Medicines and excipients across the compendia with new technologies, incorporate safety advancements, and address specificity for impurity testing.**

**In this work, we present modernization of three USP drug product monographs for naphazoline hydrochloride nasal and ophthalmic solutions, and for naphazoline hydrochloride and pheniramine maleate ophthalmic solution[1-3]. A single LC method is developed for the analysis of the active pharmaceutical ingredients (APIs) and their corresponding related compounds.**

| Compounds | | Formula | Monoisotopic mass (m/z) |
|---|---|---|---|
| Pheniramine maleate API & related compounds | Pheniramine maleate | $C_{20}H_{24}N_2O_4$ | Free base: 356.2 |
| | 2-benzylpyridine | $C_{12}H_{11}N$ | 169.1 |
| | 4-benzylpyridine | $C_{12}H_{11}N$ | 169.1 |
| Naphazoline hydrochloride API & related compounds | Naphazoline HCl | $C_{14}H_{15}ClN_2$ | Free base: 246.1 |
| | 1-naphthylacetic acid | $C_{12}H_{10}O_2$ | 186.1 |
| | Related comp. A | $C_{14}H_{16}N_2O$ | 228.1 |

*Table 1. APIs and their related compounds for USP modernization.*

## METHODS

### Sample Preparation

Standard stock solutions were prepared in diluent (90:10 mobile phase A/mobile phase B) and subsequently diluted to make a resolution mixture with 100 µg/mL of each compound, working standard with 500/40 µg/mL of pheniramine maleate/naphazoline HCl, and linearity standard solutions.

### LC Method

| | |
|---|---|
| LC System | ACQUITY Arc with PDA & ACQUITY QDa Mass Detector |
| Solvents | A: 0.05% Triethylamine & 0.05% phosphoric acid in water<br>B: 0.05% Phosphoric acid in acetonitrile |
| Column | XSelect CSH $C_{18}$ (4.6 x 150 mm, 5 µm) |
| Flow Rate | 2.0 mL/min |
| Column Temp. | 40 °C |
| Injection Vol. | 8.0 µL |
| Sample Temp. | 10 °C |
| Gradient | Time / Flow (mL/min) / %A / %B<br>1 Initial 2.000 95.0 5.0<br>2 6.00 2.000 95.0 5.0<br>3 13.00 2.000 5.0 95.0<br>4 14.50 2.000 5.0 95.0<br>5 14.60 2.000 95.0 5.0<br>6 19.00 2.000 95.0 5.0 |
| PDA Detection | 200 – 400 (derived at 280 nm) |
| MS Detection | Ionization mode: ESI+, ESI-<br>MS Acquisition range: 100 - 250 Da<br>Probe temperature: 600 °C<br>Split ratio: 1:100<br>Makeup solvent: 0.1% ammonium hydroxide in 90:10 water/methanol |
| Wash solvents | Purge/sample wash: 80/20 water/methanol<br>Seal wash: 90:10 water/acetonitrile |

*Table 2. Conditions of the final method.*

## RESULTS AND DISCUSSION

### Method Development

Columns with a wide range of selectivities were screened with acetonitrile and methanol solvents, under low and high conditions. Method with best separation was optimized by evaluating the effect of gradient slope, column temperature, pH, flow rate, and mobile phase additives.

The XSelect CSH $C_{18}$ column with a low pH of 2.7 (adjusted with formic acid) and acetonitrile solvent produced acceptable and robust separation for the analysis of active ingredients and their related substances (Figure 1). Mass spectra data acquired using an ACUQITY QDa Detector was used to identify the peaks by their detected masses.

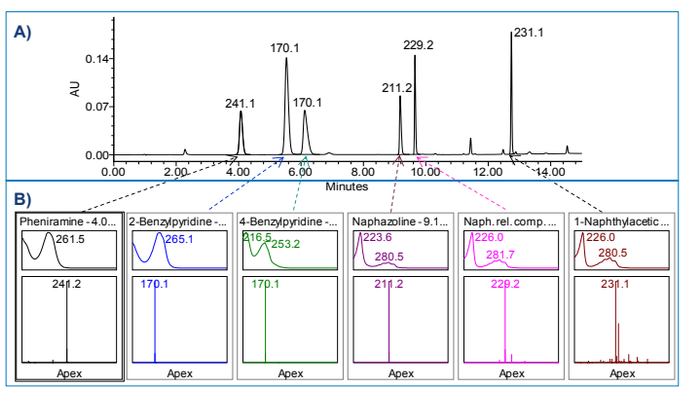



*Figure 1. Resolution mixture run on XSelect CSH $C_{18}$ column with 0.2% formic acid in water and acetonitrile (A). Mass analysis window from the Empower software displays PDA and MS spectral data in one plot (B). UV at 260 nm.*

Analysis of a working standard solution with 500/40 µg/mL of pheniramine maleate/naphazoline HCl showed a USP tailing of 2.2 for pheniramine API. Addition of triethylamine (TEA) ion-pairing reagent to the mobile phase reduced peak tailing to 1.5 by minimizing secondary interaction of the analyte with the stationary phase (Figure 2).

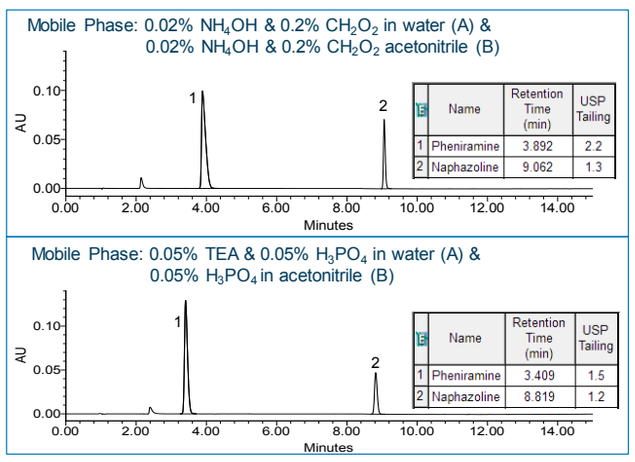
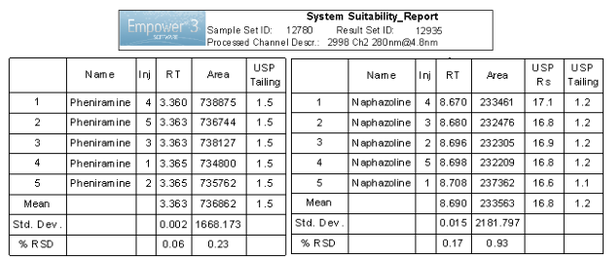



*Figure 2. Mobile phase optimization. Addition of triethylamine (TEA) reduced tailing of pheniramine API peak in a working standard solution. XSelect CSH $C_{18}$ with optimized wavelength to 280 nm for API assay.*

### System Suitability

System suitability results of five replicate injections of the working standard showed excellent repeatability of retention times and peak areas (Figure 3).




| | Name | Inj | RT | Area | USP Tailing |
|---|---|---|---|---|---|
| 1 | Pheniramine | 4 | 3.360 | 738875 | 1.5 |
| 2 | Pheniramine | 5 | 3.363 | 736744 | 1.5 |
| 3 | Pheniramine | 3 | 3.363 | 738127 | 1.5 |
| 4 | Pheniramine | 1 | 3.365 | 734800 | 1.5 |
| 5 | Pheniramine | 2 | 3.365 | 735762 | 1.5 |
| Mean | | | 3.363 | 736862 | 1.5 |
| Std. Dev. | | | 0.002 | 1668.173 | |
| % RSD | | | 0.06 | 0.23 | |

| | Name | Inj | RT | Area | USP Rs | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Naphazoline | 4 | 8.670 | 233461 | 17.1 | 1.2 |
| 2 | Naphazoline | 3 | 8.680 | 232476 | 16.8 | 1.2 |
| 3 | Naphazoline | 2 | 8.696 | 232305 | 16.9 | 1.2 |
| 4 | Naphazoline | 5 | 8.698 | 232209 | 16.8 | 1.2 |
| 5 | Naphazoline | 1 | 8.708 | 237362 | 16.6 | 1.1 |
| Mean | | | 8.690 | 233563 | 16.8 | 1.2 |
| Std. Dev. | | | 0.015 | 2181.797 | | |
| % RSD | | | 0.17 | 0.93 | | |

*Figure 3. System suitability results for 5 replicate injections of working standard solution.*

### Linearity of APIs

Linearity evaluated from 80 to 120% range with respect to the API concentration in a working concentration showed correlation coefficient (R2) greater than 0.999 (Figure 4).

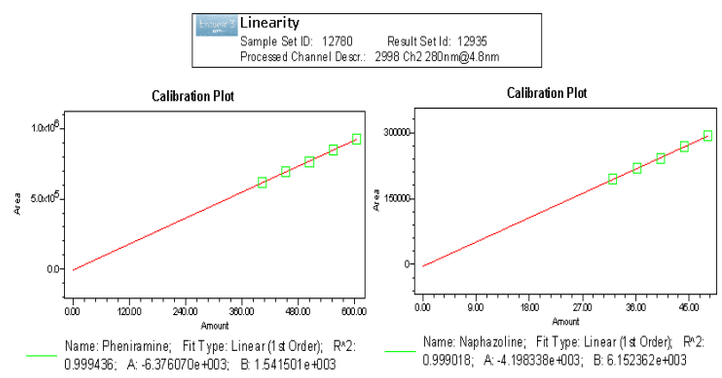



*Figure 4. Linearity for pheniramine and naphazoline APIs.*

### Related compounds

Separation between active ingredients and their related compounds showed a minimum USP resolution of 2.2 (Figure 5).

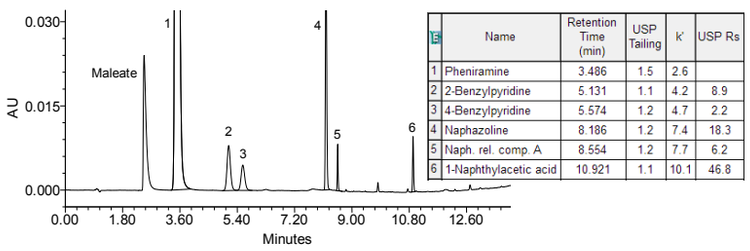



*Figure 5. Working sample spiked with related compounds to show separation for all analytes. UV at 260 nm.*

Sensitivity for related compounds was demonstrated by measuring signal-to-noise at 0.1% level with respect to the pheniramine and naphazoline APIs concentration in a working sample solution (Figure 6). Optimizing the injection volume and detection wavelength for related compounds of naphazoline API provided a S/N of 36 and 33, respectively.

A)

| Peak ID | Related compound | Conc. (ug/mL) | Inj. Vol. (uL) | Detection (nm) | USP S/N |
|---|---|---|---|---|---|
| 1 | 2-benzylpyridine | 0.52 | 8 | 260 | 46 |
| 2 | 4-benzylpyridine | 0.50 | 8 | 260 | 25 |
| 3 | Naphazoline related comp. A | 0.042 | 45 | 280 | 36 |
| 4 | 1-Naphazoline acid | 0.041 | 45 | 280 | 33 |

B)

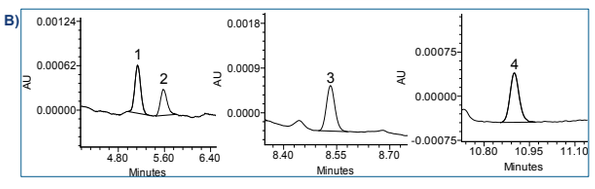



*Figure 6. S/N values for related compounds at 0.1% level with respect to the pheniramine and naphazoline APIs in a working sample solution (A). Related compounds at 0.1% level (B).*

### Analysis of ophthalmic and nasal solutions

Commercially available ophthalmic and nasal solutions were analyzed using the developed method.

Sample solutions were prepared by dilution in the diluent (90:10 mobile phase A/mobile phase B) to the working concentrations:

- 500 µg/mL pheniramine maleate/40 µg/mL naphazoline HCl for Visine-A, Naphcon-A, Opcon-A eye allergy relief solutions
- 40 µg/mL naphazoline HCl for Walgreens, Clear eyes redness and cooling eye drops and Sato Nazal Spray

Spectral purity or homogeneity of the active ingredients was confirmed using peak purity tools in the Empower Software (Figure 7). Using both UV and MS spectral data enabled spectral homogeneity determination within the sample solutions.

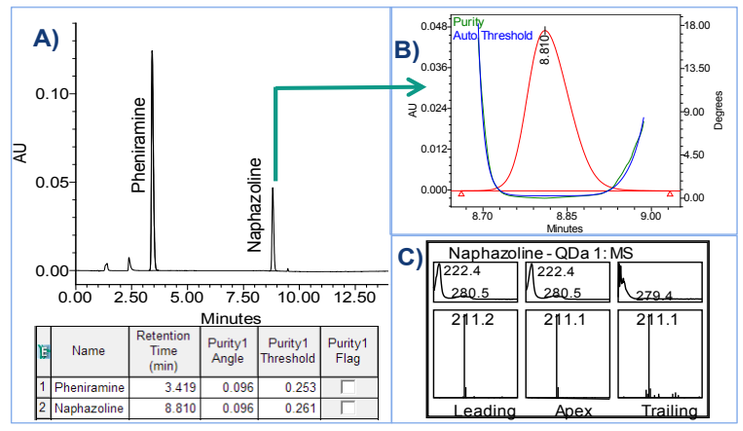



*Figure 7. Peak purity determination in Visine sample, UV at 280 nm . The APIs purity angles are below threshold angles, confirming spectral homogeneity (A). UV peak purity plot (B) and MS peak purity spectrum (C) for naphazoline.*

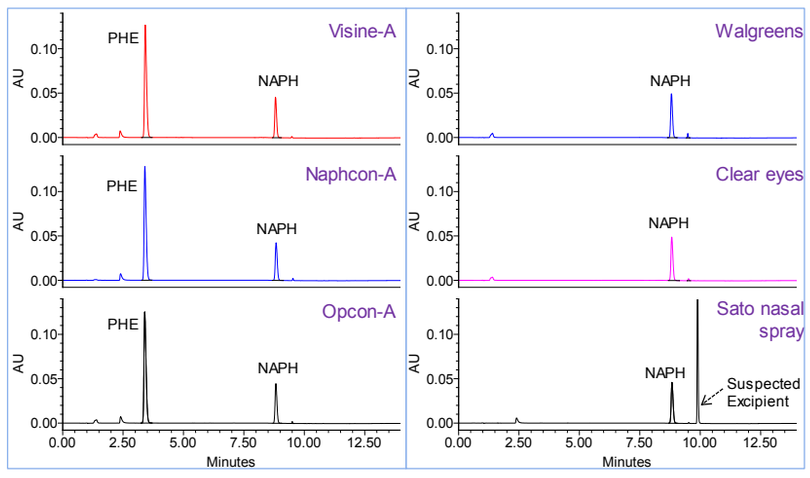



*Figure 8. Ophthalmic and nasal solutions. % Recovery of pheniramine (PHE) and naphazoline (NAP) APIs were within the USP assay of 90 - 110% listed in the USP monographs[1-3].*

## CONCLUSION

- A single LC method, specific for analysis of active ingredients and their related compounds was developed to modernize three USP monographs for naphazoline HCl and pheniramine maleate ophthalmic and nasal solutions
- The ACQUITY QDa enabled quick identification of analytes by mass detection and confirmation of spectral peak purity within the sample solution.

### References

1. USP Monograph, Naphazoline Hydrochloride Nasal Solution, USP40-NF35, The United States Pharmacopeia Convention, official December 2017.
2. USP Monograph, Naphazoline Hydrochloride Ophthalmic Solution, USP40-NF35, The United States Pharmacopeia Convention, official December 2017.
3. USP Monograph, Naphazoline Hydrochloride and Pheniramine Maleate Ophthalmic Solution, USP40-NF35, The United States Pharmacopeia.

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