MODERNIZATION OF USP MONOGRAPHS FOR NAPHAZOLINE HYDROCHLORIDE AND PHENIRAMINE MALEATE OPHTHALMIC AND NASAL SOLUTIONS

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Authors Margaret Maziarz(1), Sujatha Ramakrishna(2), Paul Rainville(1), Sherri Naughton(1), Yukari Haramaki (3)
Affiliations (1) Waters Corporation, MA, USA, (2) U.S. Pharmacopeial Convention, MA, USA (3) Waters Asia Pacific, Tokyo, Japan

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

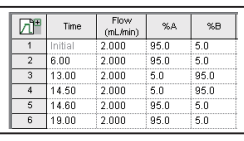
LC System	ACQUITY Arc with PDA & ACQUITY QDa Mass Detector
Solvents	A: 0.05% Triethylamine & 0.05% phosphoric acid in water B: 0.05% Phosphoric acid in acetonitrile
Column	XSelect CSH C18 (4.6 x 150 mm, 5 µm)
Flow Rate	2.0 mL/min
Column Temp.	40 °C
Injection Vol.	8.0 µL
Sample Temp.	10 °C
Gradient	
PDA Detection	200 – 400 (derived at 280 nm)
MS Detection	Ionization mode:ESI+, ESI- MS Acquisition range: 100 - 250 Da Probe temperature: 600°C Split ratio: 1:100 Makeup solvent: 0.1% ammonium hydroxide in 90:10 water/methanol
Wash solvents	Purge/sample wash: 80/20 water/methanol 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 C18 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

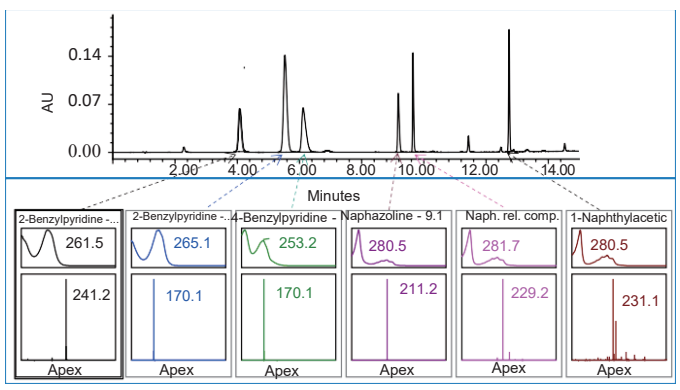


Figure 1. Resolution mixture run on XSelect CSH C18 column with 0.2% formic acid in water and acetonitrile (A). Mass analysis window from the Empower software displays PDA and MS spectral data in one plot (B). UV at 260 nm

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).

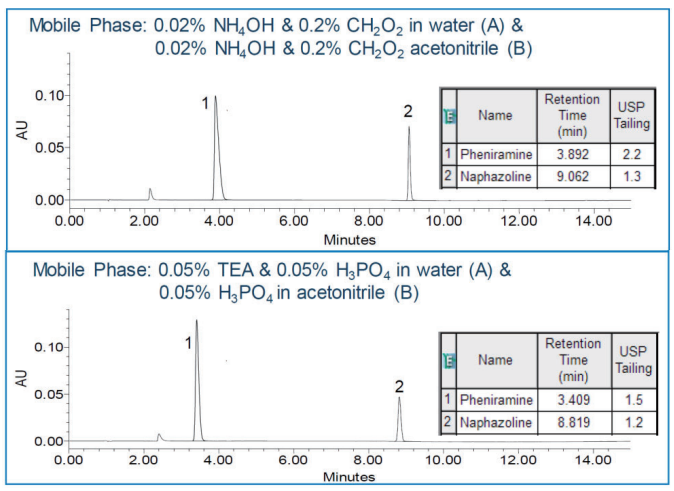
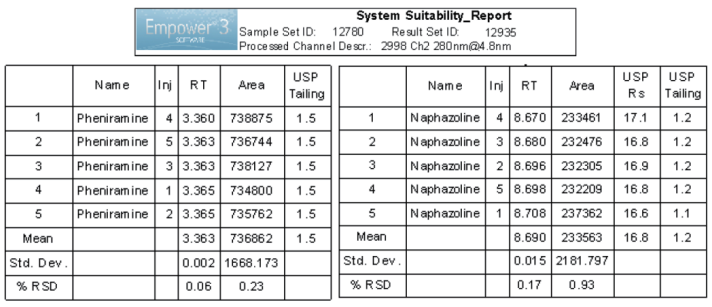


Figure 2. Mobile phase optimization. Addition of triethylamine (TEA) reduced tailing of pheniramine API peak in a working standard solution. XSelect CSH C18 with optimized wavelength to 280 nm for API assay

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).

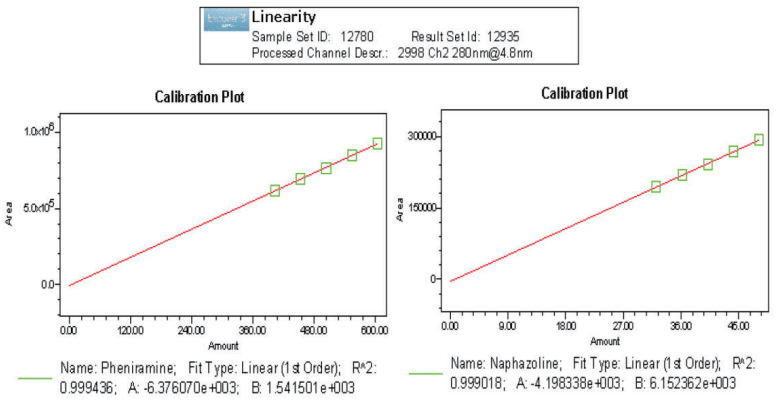


Figure 4. Linearity for pheniramine and naphazoline APIs.

Related compounds

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

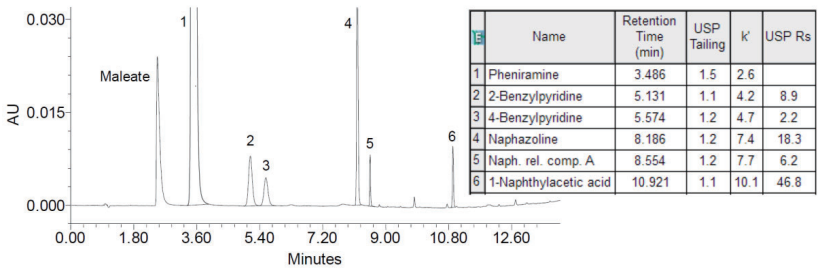


Figure 5. Working sample spiked with related compounds to show separation for all analytes. UV at 260 nm.

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)

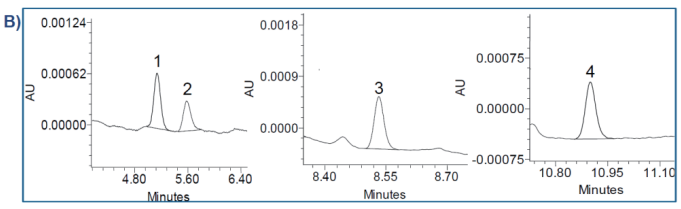


Figure 6. S/N values for related compounds at 0.1% level with respect to the pheniramine and naphazoline APIs in a working sample solution (A). Related compounds at 0.1% level (B)

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.

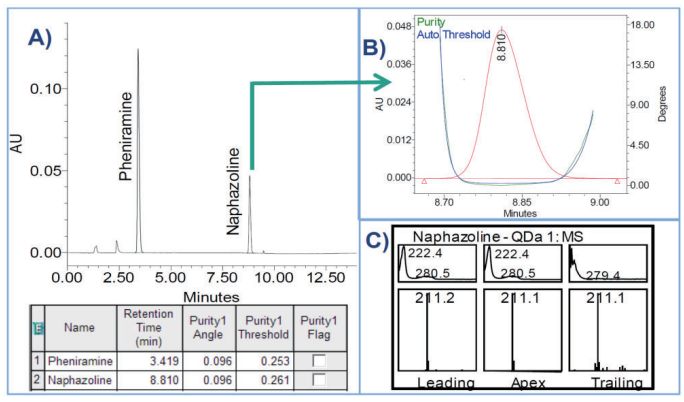


Figure 7. Peak purity determination in Visine sample, UV at 280 nm The APIs purity angles are below threshold angles, confirming spectral homogeneity (A). UV peak purity plot (B) and MS peak purity spectrum (C) for naphazoline

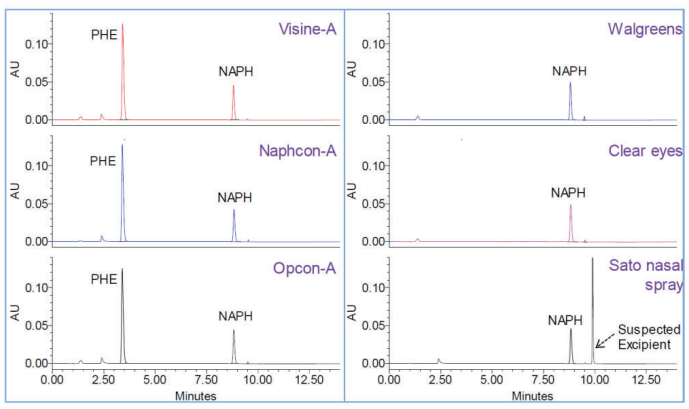


Figure 8. Ophthalmic and nasal solutions. % Recovery of pheniramine (PHE) and naphazoline (NAP) APIs were within the USP assay of 90 - 110% listed in the USP monographs1-3. UV at 280 nm

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-NF35The 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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