T1330-09-57

SIMULTANEOUS DETERMINATION OF NAPHAZOLINE HYDROCHLORIDE AND PHENIRAMINE MALEATE ALONG WITH THEIR RELATED COMPOUNDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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aaps PharmSci 360

PURPOSE

In recent years, the United States Pharmacopeia (USP) has undertaken a modernization effort to update outdated analytical methodologies in its monographs. This initiative aims to provide updated public standards and reinforce regulatory agencies' efforts to safeguard public health. The focus is on the main sections of monographs, which include identification, assay, and organic impurities. A key element of the modernization process is the elimination of hazardous solvents and reagents in the analytical procedure. Currently, the industry uses separate chromatographic methods to analyze each API in pharmaceutical formulations. While effective, this approach can generate large amounts of hazardous waste from organic solvents.

To minimize hazardous waste, one solution is to use a single chromatographic method to analyze multiple active materials and their related compounds. In this study, we demonstrate the combination of three USP chromatographic methods into a single LC method for analyzing two APIs (naphazoline hydrochloride and pheniramine maleate) and their related compounds [1-3]. Names and chemical of these analytes are detailed in Table 1. formulas

Table 1. Names and molecular formulas of the APIs and related compounds that were used in this study.

Compounds		Formula
Pheniramine maleate API & related compounds	Pheniramine maleate	$C_{20}H_{24}N_2O_4$
	2-benzylpyridine	$C_{12}H_{11}N$
	4-benzylpyridine	$C_{12}H_{11}N$
Naphazoline hydrochloride API & related compounds	Naphazoline HCl	$C_{14}H_{15}ClN_2$
	1-naphthylacetic acid	$C_{12}H_{10}O_2$
	Related comp. A	$C_{14}H_{16}N_2O$

METHODS

Sample Preparation

Pheniramine maleate and its related compounds (2-benzylpyridine, 4-benzylpyridine) were kindly provided by the United States Pharmacopeia (USP) (Rockville, MD, USA). Naphazoline HCl and its related compounds (1-naphthylacetic acid, Related comp. A) were also each provided by the USP. Standard stock solutions were prepared in diluent (90:10 mobile phase A/mobile phase B) and subsequently diluted to make a resolution mixture that contains pheniramine/naphazoline 500/40 ug/mL with 5 ug/mL related substances. All solutions were stored in PP containers in a freezer (-20 °C). Over the counter ophthalmic solutions formulations containing 0.025% (v/v) of pheniramine maleate and 0.3% v/v naphazoline HCl were purchased from a local drug store.

LC Method

LC System:	Alliance™ iS HPLC System with a Tunable UV detector
Detection:	TUV (Dual Wavelength, 260 and 280 nm)
Column:	5 µm, 4.6×150 mm XSelect™ CSH C_{18} Column, pH range: 2-10
Column Temp.:	40 °C
Sample Temp.:	5 °C
Injection Volume:	8 µL
Flow Rate:	2.0 mL min^{-1}
Mobile Phase A:	0.05% (v/v) triethyl amine and 0.05% (v/v) phosphoric acid in water (non pH adjusted)
Mobile Phase B:	0.05% (v/v) phosphoric acid in Acetonitrile
Gradient Profile:	Initial hold of 6 minutes at 5% organic and 95% aqueous followed by a linear gradient of organic from 5-95% over 7 minutes.

RESULTS

System Suitability and Precision

To verify the functionality of the chromatographic system, it was important to perform System Suitability Testing (SST). SST is a standard procedure used to verify the efficiency and repeatability of a chromatographic system to ensure its suitability for a specific analysis. To demonstrate this, the system underwent 12 replicate injections of the SST working standard (500/40 µg/mL of pheniramine maleate/naphazoline HCl), and the results, presented in Table 2, showed that the relative standard deviation (%RSD) for the peak areas of naphazoline and pheniramine was less than 0.1 for 12 consecutive injections. The %RSD for the retention time for these two peaks was 0.02 for pheniramine and 0.14 for naphazoline. These findings indicate that the developed method and the system offer outstanding repeatability of retention times and peak

Table 2. System suitability results for 12 replicate injections of working standard solution.

Injection	RT	Area	Injection	RT	Area
1	8.502	139752	1	4.543	2152529
2	8.509	139833	2	4.557	2151601
3	8.504	139831	3	4.547	2153481
4	8.505	139713	4	4.547	2151214
5	8.505	140127	5	4.552	2153755
6	8.504	139939	6	4.541	2152935
7	8.505	139705	7	4.550	2154672
8	8.503	139993	8	4.543	2153289
9	8.505	140010	9	4.552	2155339
10	8.504	139981	10	4.540	2152494
11	8.504	139934	11	4.535	2154027
12	8.501	139780	12	4.544	2154806
Mean	8.504	139883	Mean	4.546	2153345
Std. Dev.	0.002	133.868	Std. Dev.	0.006	1267.523
% RSD	0.02	0.10	% RSD	0.14	0.06

Linearity of APIs

In this study, linearity was assessed by preparing five mixtures \ at concentrations ranging from 80% to 120% of the target concentration of 500/40 µg/mL of pheniramine maleate/naphazoline HCl.
Each solution was then injected in duplicate into the chromatographic system, and the response area was recorded. The resulting linear calibration curves were constructed by plotting peak area against concentration, and regression equations were computed, indicating a strong correlation coefficient (R2) greater than 0.997, as shown in Figure 1.

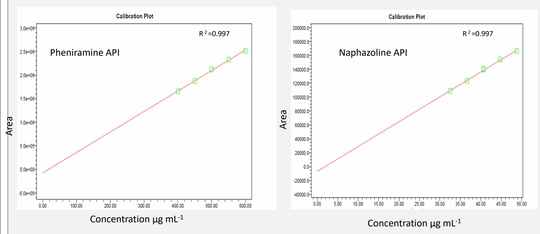


Figure 1. Linearity for pheniramine and naphazoline APIs. The curves were constructed by injecting five levels of the working standard at concentrations ranging from 80% to 120% of the target concentration.

Related compounds

To assess the developed method's capability in separating the active ingredients from their related compounds, it was interesting to run the method on a standard Resolution Mixture that contains naphazoline hydrochloride/ pheniramine maleate and their related compounds. The obtained results demonstrated that the method effectively separated all the compounds in the mixture, with a minimum USP resolution of 2.4, as depicted in Figure 2 and Table 3.

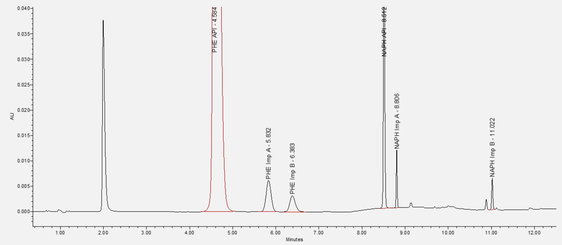


Figure 2. Working sample spiked with related compounds at 5% concentration levels of the APIs. The final solution contained : 500 µg/mL pheniramine (25 µg/ mL of its related compounds, Phe Imp A, Phe imp B) , and 40µg/mL naphazoline (2 µg/mL Naph Imp A and Naph Imp B). Single wavelength UV absorption at at 260 nm

Table 3. USP resolution values for all the peaks of the compounds in the Resolution Mixture (Figure 3).

		Retention Time	K Prime	USP Resolution	USP Resolution (HH)
1	PHE API	4.584	3.6		
2	PHE Imp A	5.832	4.8	5.5	5.7
3	PHE Imp B	6.383	5.4	2.4	2.5
4	NAPH API	8.521	7.5	14.2	14.6
5	NAPH Imp A	8.806	7.8	5.4	6.0
6	NAPH Imp B	11.022	10.0	42.5	48.8

Intra-day and inter-day Precisions

Intra-day precision of the method was evaluated by performing 12 replicate injections of the system suitability mixture as previously demonstrated in the system suitability section. For inter-day precision, the same samples were analyzed in two different days (12 replicate injections on day one and additional five replicate injections on the second day). Results are displayed in Figure 3.

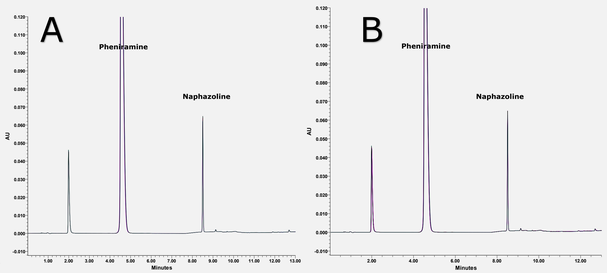
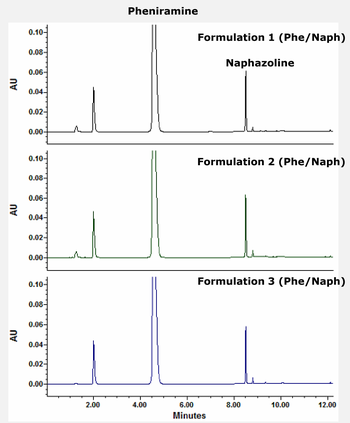


Figure 3. Representative separation of a System Suitability Test (SST) solution: A. 12 replicate injections of the solution on the same day and B represents an overlay of 17 injections of the same solution over two days. The SST solution contained (500/40 µg/mL of pheniramine maleate/naphazoline HCl). Conditions are the same as in experimental section (UV at 280 nm).

Analysis of Ophthalmic and Nasal Solutions

Application of the method to the analysis of samples obtained from commercially available ophthalmic and nasal solutions was then performed. The samples were prepared as follows: the solutions were diluted in the diluent (90:10 mobile phase A/mobile phase B) to the working concentrations of 500 µg/mL pheniramine maleate/40 µg/mL naphazoline HCl for formulas 1, 2, and 3 eye allergy relief solutions and 40 µg/mL naphazoline HCl for formulas 4, 5, and 6 redness and cooling eye



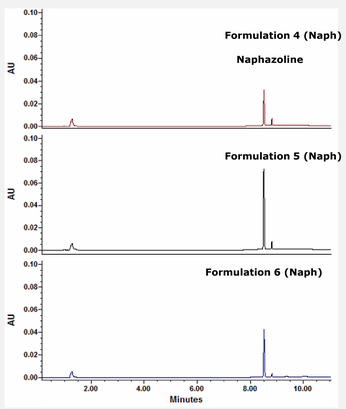


Figure 4. Representative separation of commercially available nasal solutions that contain pheniramine (PHE) and naphazoline APIs (formulations 1, 2, and 3) as well as solutions that contain naphazoline API only (formulations 4, 5, and 6).

CONCLUSIONS

- A single LC method, specific for analysis of active ingredients and their related compounds was developed to combine three USP monographs for naphazoline HCl and pheniramine maleate ophthalmic and nasal solutions.
- Alliance iS HPLC System enabled rapid and reliable separation and quantification of multiple APIs along with their related compounds in a single HPLC method.

REFERENCES

11] 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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