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High Performance Liquid Chromatography Analysis of Tetrahydrozoline Hydrochloride in Ophthalmic Solution by Silica Column Eluted with Aqueous Solvent Mixtures

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ABSTRACT

A HPLC method using bared silica column eluted with aqueous solvent mobile phase was developed for determination of tetrahydrozoline hydrochloride in ophthalmic preparations. A mixture of methanol and water (70:30, v/v) containing 0.03% triethylamine and 0.02% acetic acid was used as mobile phase, and chlorpheniramine maleate as an internal standard. The flow rate was 1 mL/min and the detection was at 254 nm. The quantitation limit was 1.0 $\mu\text{g/mL}$. Average recoveries range from 98.9 to 99.9%. The linearity of the calibration curve of tetrahydrozoline hydrochloride was well correlated ($r^2 = 0.9996$) within the range from 12.5 to 500 $\mu\text{g/mL}$ as well as from 1.0 to 20 $\mu\text{g/mL}$ ($r^2 = 0.9997$). This study further reports a simple and quick method for routine quantitative analysis of tetrahydrozoline hydrochloride in an ophthalmic solution that contains a relatively high concentration of sulfamethoxazole sodium and methyl paraben.

Key words: tetrahydrozoline, ophthalmic solution, analysis, HPLC, silica column

INTRODUCTION

Tetrahydrozoline hydrochloride (TH) is an adrenergic agent (vasoconstrictors) and derived from imidazoline. This compound is closely related to naphazoline hydrochloride in its pharmacological action. When applied topically to the nasal mucosa, TH causes vasoconstriction that results in reduction of local swelling and congestion. It is also used in a 0.05% solution as an ocular decongestant. Applied as a 0.05 or 0.1% solution as nasal drops or 0.05% solution as eye drops, TH nasal solution and ophthalmic solution are admitted in USP XXIV.

A number of methodologies are available for analysis of TH in nasal solution or ophthalmic solution. They include colorimetric (USP XXIV)⁽¹⁾, HPLC methods with octadecylsilyl silica (ODS) column⁽²⁻⁴⁾ and ion-pairing technique^(5,6), ODS column with buffering mobile phase^(7,8), and gas chromatography (GC)⁽⁹⁾. The colorimetric method is tedious and insensitive for regular use. Recently, reversed-phase HPLC (RP-HPLC) has gained great appreciation for analysis of pharmaceutical substances. A large number of drugs possess a basic character, which are conveniently analyzed when running RP-HPLC on chemically bonded phases such as ODS. However, it has been shown that basic compounds with a nitrogenous moiety often exhibit an unsatisfactory degree of peak tailing on bonded-phase packing materials. Adding anti-tailing agents such as amine modifiers or ion-pair reagents may reduce the problem of tailing. Although it can improve the peak tailing, as more salts are

added to the mobile phase, injuries to the columns and instrumental tubing can be expected. In addition, it takes more time to prepare the mobile phase and rinse the columns and tubing after the assay is finished. Furthermore, the concentration of active ingredients in pharmaceutical preparations differs in a wide range. It is difficult to simultaneously analyze the desired ingredients with a wider difference in concentration.

This study describes a simple, quick and novel application of bared silica column. It offers a powerful and versatile approach to analyze an ophthalmic solution that is formulated with a low concentration of TH with the presence of a high concentration of sulfamethoxazole sodium (SM) and methylparaben (MP).

MATERIALS AND METHODS

I. Materials and Reagents

Tetrahydrozoline hydrochloride ophthalmic solution (contains sulphamethoxazole sodium) was provided by Wu Fu Laboratories Co., Ltd (Taipei, Taiwan). LC grade methanol was obtained from LAB-SCAN (Dublin Ireland). Triethylamine and acetic acid were the products of Merck (Darmstadt Germany). TH and chlorpheniramine maleate (CM) (internal standard) were the working standard provided by National Laboratories of Foods and Drugs, Taiwan, R.O.C.

II. HPLC Apparatus and Conditions

HPLC analysis was conducted with a Waters model of 510 solvent delivery pump with UV-VIS detector and U6K

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injector. A LiChroCART 250-4 HPLC-Cartridge (Merck, Darmstadt Germany) LiChrospher Si 60 (5 μm) column (Merck, Darmstadt Germany) was applied. The silica gel column was initially preserved in hexane. Before analysis, a serial recondition was conducted by eluting with solvent of gradually increasing polarity from ethyl acetate, dichloromethane, acetonitrile, methanol, and then water in the final (the volume for each solvent is approximately equal to 100 mL). Thereafter, this silica gel column can be eluted with the use of reversed-phase eluents. The mobile phase is consisted of a mixture (methanol:water = 7:3, v/v) that contains 0.03% (v/v) of triethylamine and 0.02% (v/v) of acetic acid. The flow rate is 1.0 mL/min, injection volume is 20 μL and the detection is at 254 nm.

III. Preparation of Standard Solutions

To examine the linearity of the assay, the calibration curve for TH at concentrations ranged from 12.5 to 500 $\mu\text{g}/\text{mL}$ or from 1.0 to 20 $\mu\text{g}/\text{mL}$ in mobile phase was prepared. Standard solutions containing TH were added with 8 $\mu\text{g}/\text{mL}$ of CM. The peak area ratio (PAR) of TH to CM was measured and the calibration curve was obtained from the least-squares linear regression of the PAR versus concentrations.

IV. Preparation of Sample Solutions

Six samples of commercial tetrahydrozoline hydrochloride ophthalmic solutions containing SM were employed for quantitation. One of the samples was diluted with mobile phase to obtain 10 $\mu\text{g}/\text{mL}$ of TH. The others were directly filtered through a 0.45 μm membrane before HPLC analysis.

V. Recovery

The three concentration of TH, 20, 40, 50 $\mu\text{g}/\text{mL}$ was spiked into three individual samples. The recovery of TH was determined by assaying samples with known concentrations of TH. Three replicates of HPLC analysis were examined for each sample, and the individual and the mean value were reported.

VI. Validation of Assay Method

Several blank samples containing excipients were tested for the absence of interfering compounds. The intra- and inter-assay coefficients of variation and standard deviations of mean were used to validate the precision and accuracy of the assay by determining standard samples of TH. For inter-day validation, six sets of control samples at six different concentrations (12.5-500 $\mu\text{g}/\text{mL}$ or 1.0-20 $\mu\text{g}/\text{mL}$) were evaluated on six different days (six standard curves were constructed). For intraday validation, six sets of controls at six different drug concentrations were assayed with one standard curve on the same run.

VII. Quantification and Calibration Curve Preparation

The calibration curve was constructed for Quantitation. TH in various matrices were prepared. Standard samples containing TH were added with 8 $\mu\text{g}/\text{mL}$ CM and analyzed as described above. The PAR of TH to CM was measured and the calibration curve was obtained from the least-squares linear regression of the PAR versus spiked concentrations. The regression line was used to calculate the concentrations of TH in the unknown samples based on the PAR.

RESULTS

Table 1 illustrates the formulation of interested ophthalmic solution. The concentration of SM, TH, and MP are 2% (w/v), 0.0025% (w/v) and 0.1% (w/v), respectively. The ultraviolet characters of those ingredients are shown in Table 2.⁽¹⁰⁾ By estimating from the characteristics of UV absorption and concentration in the formulation, the detectivity of SM was 5,600 times higher than that of TH when using ODS-HPLC method for assaying TH. This widely difference in concentration in pharmaceutical preparations could cause more difficulty in separating TH from SM by RP-HPLC methods. In practice, analysis by the way of Inertsil 10 ODS column (GL Sciences Inc., Tokyo Japan) with mobile phase of water:methanol:acetonitrile (=1:5:5)⁽²⁾, the small peak of TH was covered by an over scale peak of SM (Figure 1). Regarding ion-pairing technique by using Inertsil 5 C₈ column (4.6 \times 150 mm), the mobile phase containing 10% of 5 mM Sodium octane sulphonate - 5 mM Na₂HPO₄ (pH 7) in acetonitrile-methanol (1:1,v/v)⁽⁶⁾ was desirable in the analysis of TH in the same sample matrix. However, the small peak corresponding to TH can be well separated from the main peak by means of the ion-pairing technique at only appropriate pH value as shown in Figure 2.

The addition of triethylamine (TEA) or equivalents in the mobile phase plays as a masking agent for free silanol

Table 1. The formula of tetrahydrozoline HCl ophthalmic solution

Ingredients	Concentration (mg/mL)
Sulfamethoxazole Sodium	20.000
Tetrahydrozoline HCl	0.025
Methyl Paraben	1.000
Borax	1.910
Boric Acid	1.240
Sodium Thiosulfate	2.000
Borneol	5.000
L-Menthol	0.050

Table 2. The ultraviolet character^a of interested ingredients in tetrahydrozoline HCl ophthalmic solution

Ingredients	Ultraviolet Character
Tetrahydrozoline	265 nm ($A_1^1 = 21$, in aqueous acid)
	272 nm, no alkaline shift
Sulfamethoxazole	265 nm ($A_1^1 = 175$, in aqueous acid)
	256 nm ($A_1^1 = 673$, in aqueous alkali)
Methylparaben	257 nm ($A_1^1 = 1075$, in ethanol)

A_1^1 : specific absorbance (abbreviation of $A_1^{1\%_{1\text{cm}}}$).

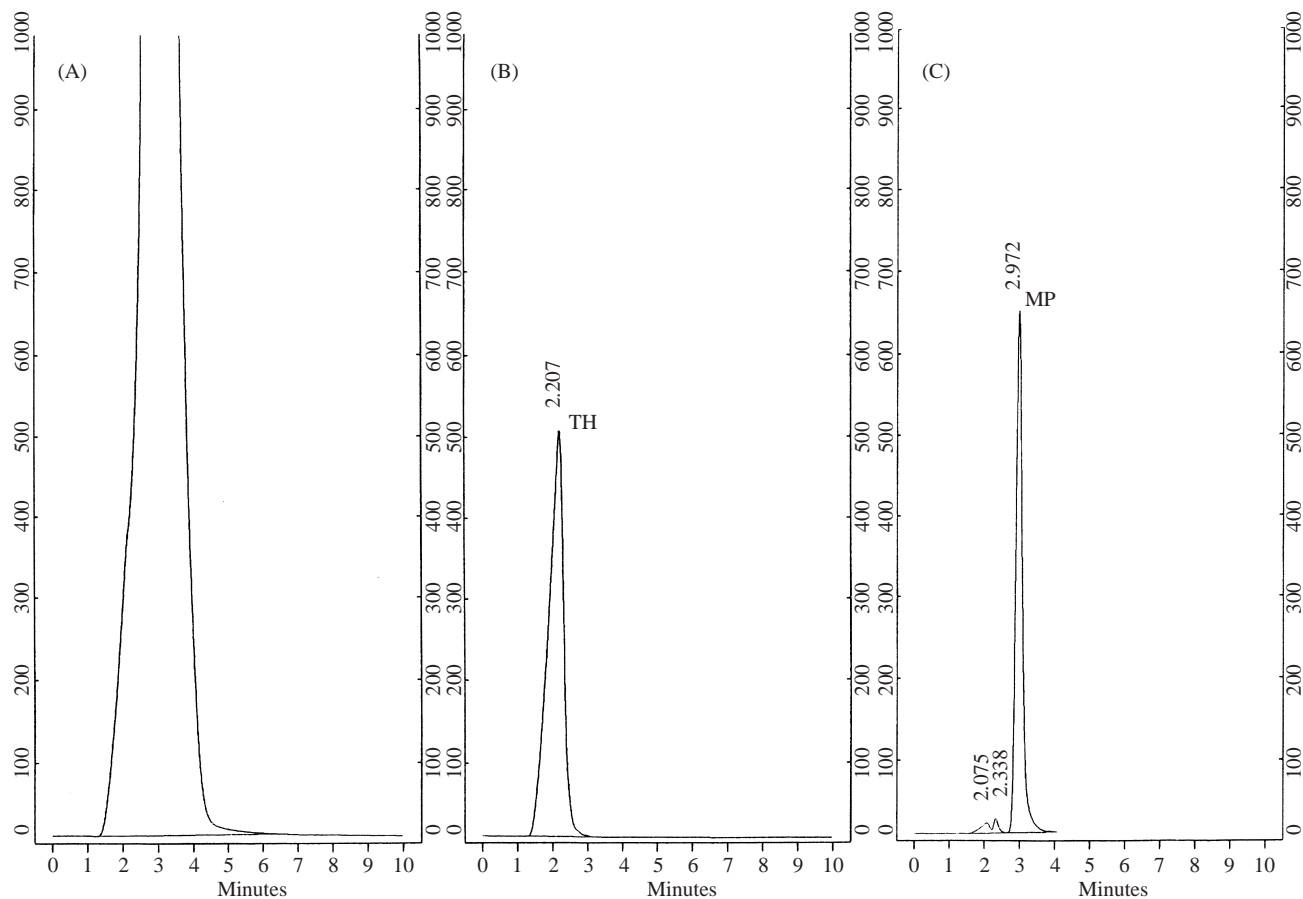


Figure 1. Chromatogram of tetrahydrozoline HCl ophthalmic solution analyzed by traditional ODS column method. A: sample solution; B: tetrahydrozoline HCl (1000 $\mu\text{g/mL}$); C: methylparaben (50 $\mu\text{g/mL}$).

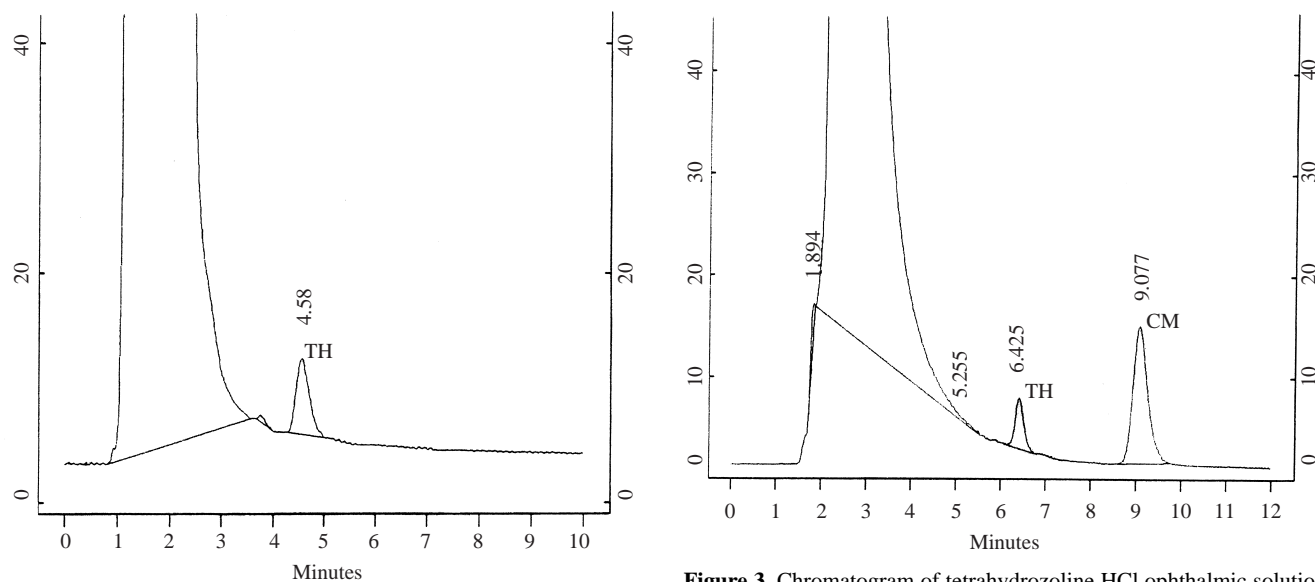


Figure 2. Chromatogram of tetrahydrozoline HCl ophthalmic solution analyzed by ion-pairing method. (TH:25 $\mu\text{g/mL}$).

Figure 3. Chromatogram of tetrahydrozoline HCl ophthalmic solution analyzed by silica column eluted with aqueous solvent mixture (TH:10 $\mu\text{g/mL}$; CM:8 $\mu\text{g/mL}$).

group on silica gel to minimize the interaction between basic drugs and silica gel, which generally causes the tailing of peak and also results in a longer retention time. In this study, it was found that the addition of TEA at a level elucidated in

the Experimental Section was suitable enough to accomplish the purpose.

A HPLC method employing a silica column eluted with an aqueous solvent mixture was then applied for assaying TH

in ophthalmic solution. The HPLC running condition was described in above. Figure 3 shows a typical sample analysis of HPLC with internal standard. No interfering peaks were observed for several samples of different matrices. The retention times of TH and CM were around 6.43 and 9.08 minutes, respectively. Good separation and baselines with low background were observed. The peaks of interest were well resolved and there was no interference from excipients. Also, the symmetry of both peaks (TH and CM) was clearly indicated.

Using the peak height of TH at the same injected amount without extraction as 100%, it was found that the recovery of TH added to various matrices was almost quantitative (approaching 100%, Table 3). This indicates that the method is reproducible and suitable for the analysis of pharmaceutical samples.

The interday and intraday validations for assaying TH in pharmaceutical samples in two different concentration ranges are shown in Tables 4 and 5, respectively. The coefficients of variation (CV) of interday and intraday assays for concentration range from 12.5 to 500 $\mu\text{g/mL}$ were 1.31% to 2.70% and 1.30% to 2.55% respectively, whereas for concentration range of 1.0 to 20 $\mu\text{g/mL}$ were 0.45% to 4.30% and 1.39% to 11.58%, respectively indicating that the analysis has good

Table 3. Recoveries of tetrahydrozoline HCl spiked into commercial ophthalmic solution

Spiked level ($\mu\text{g/mL}$)	Recovery (%)	Mean (%) \pm S.D.	C.V. (%)
	99.4		
20	98.3	98.9 \pm 0.55	0.56
	98.9		
	100.6		
40	99.6	99.7 \pm 0.85	0.86
	98.9		
	100.3		
50	99.2	99.9 \pm 0.66	0.67
	100.4		

precision. The relative error of mean (REM) of interday and intraday assays for concentration range from 12.5 to 500 $\mu\text{g/mL}$ was -2.63% to 6.26% and -4.05% to 1.97%, respectively, whereas for concentration range of 1.0 to 20 $\mu\text{g/mL}$

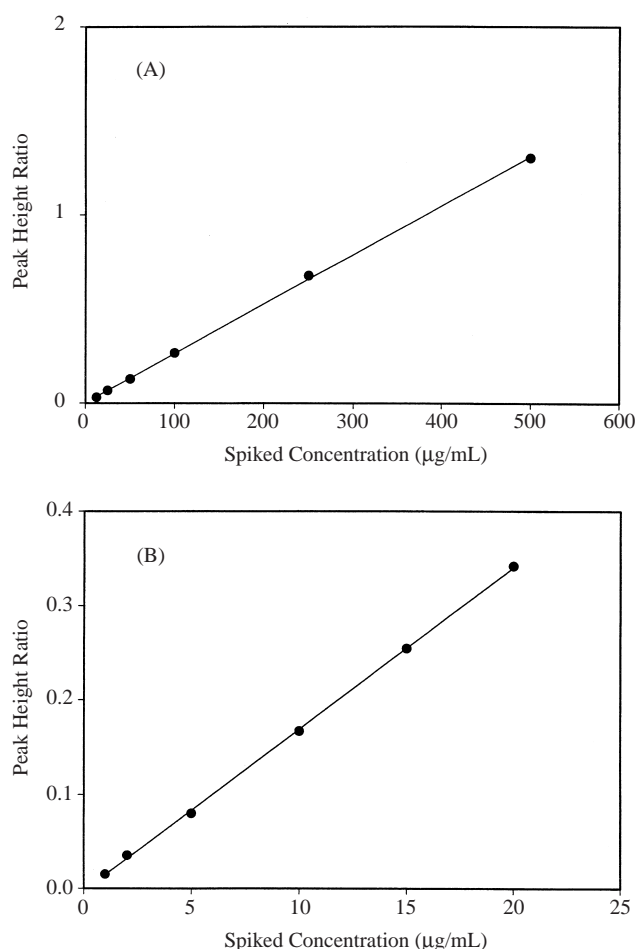


Figure 4. Calibration curve for tetrahydrozoline HCl in a concentration from 12.5 to 500 $\mu\text{g/mL}$ (A) and from 1.0 to 20.0 $\mu\text{g/mL}$ (B).

Table 4. Precision and accuracy of intraday and interday validation for concentration range from 12.5 to 500 $\mu\text{g/mL}$

Concentration ($\mu\text{g/mL}$)	Intraday			Interday		
	Mean (SD)	CV (%)	Rel Err (%)	Mean (SD)	CV (%)	Rel Err (%)
12.5	12.29(0.16)	1.30	-1.68	12.37(0.19)	1.54	-1.04
25.0	24.45(0.65)	2.55	1.80	25.57(0.43)	1.68	2.28
50.0	49.97(0.92)	1.84	-0.06	51.14(1.34)	2.62	2.28
100.0	101.97(2.09)	2.05	1.97	106.26(2.87)	2.70	6.26
250.0	244.88(4.84)	1.98	-2.05	245.92(3.21)	1.31	-1.63
500.0	479.76(10.9)	2.28	-4.05	486.86(8.16)	1.68	-2.63

SD: standard deviation; CV: coefficient of variation; Rel Err: relative error of the mean; (n=3).

Table 5. Precision and accuracy of intraday and interday validation for concentration range from 1.0 to 20 $\mu\text{g/mL}$

Concentration ($\mu\text{g/mL}$)	Intraday			Interday		
	Mean (SD)	CV (%)	Rel Err (%)	Mean (SD)	CV (%)	Rel Err (%)
1.0	0.95(0.11)	11.58	-5.00	0.93(0.04)	4.30	-7.00
2.0	1.91(0.08)	4.19	-4.50	1.95(0.07)	3.59	-2.50
5.0	5.05(0.14)	2.78	1.00	5.06(0.09)	1.78	1.20
10.0	9.78(0.14)	1.43	-2.20	9.86(0.41)	4.15	-1.40
15.0	14.86(0.24)	1.62	-0.93	14.92(0.21)	1.41	-0.53
20.0	20.14(0.28)	1.39	0.70	20.15(0.09)	0.45	0.75

SD: standard deviation; CV: coefficient of variation; Rel Err: relative error of the mean; (n=3).

were -7.00% to 1.20% and -5.00% to 1.00%, respectively depicting the high accuracy of the analysis. The linearity of the calibration curve of TH as shown in Figure 4 was well

correlated within a range from 12.5 to 500 $\mu\text{g/mL}$ (4A, $y=0.0026*x+0.0033$, $r^2=0.9996$) and from 1.0 to 20 $\mu\text{g/mL}$ (4B, $y=0.0172*x-0.0026$, $r^2=0.9997$).

DISCUSSION

The retention behavior of using silica column eluted with aqueous solvent is based on the ion-exchange-like mechanism and is controlled predominantly by the pH value⁽¹¹⁻¹³⁾. The pH of mobile phase must be chosen in such a way that the solutes are protonated and the stationary phase, silica, possesses ion-exchange capacity. As a result, basic drugs, particularly quaternary amine compounds, will be retained the most in this system. SM is a sulfonamide, a weak acidic drug with a pKa value of 5.6, and will not be protonated in this mobile phase. Therefore, a short retention time is expected. MP, an α -hydroxybenzoic acid derivative, will not interfere with assay because it can not be protonated either. TH, a tertiary amine of imidazoline derivative, can be protonated and separated from high concentrations of SM and MP due to longer retention time. Thus, the pH value is the most important parameter to control retention and separation efficiency. The increase of pH value in mobile phase increases the ion-exchanging capacity of the silica packing material. This results in the increase of capacity prolonging the retention time of basic drugs.

A typical pH effect of sample matrix on silica-base column is demonstrated on Figure 5. The pH value of an undiluted sample solution was around 8.2, and the retention time of TH was 7.02 min (Figure 5A), about one minute longer compared to that of TH standard solution (Figure 5D) whose pH value was around 7.0. The pH difference was attributed to the mixing of mobile phase and sample solution during analysis. However, this influence was minimized with the initial dilution of sample solution with mobile phase as indicated by Figures 5B and 5C.

While the pH value plays an important role in both ion-exchanging-like and ion-pairing mechanisms, how to maintain an appropriate pH value to ensure higher accuracy of assay turns out to be very critical. Compared to the ion-pairing mechanism, the ion-exchanging-like method only needs to simply mix the right volume proportions to automatically accomplish an appropriate pH value for the mobile phase. It not only reduces the complexity of procedures for mobile phase preparation but also the possibility of poor resolution.

Maintenance of equipment and column is another key factor to illustrate the superiority of the ion-exchanging-like method. Following the end of assay, the way of column cleansing impacts the maintenance of equipment. The more salts that remain in the column or tubing, the more likely damage to the instrument resulting from crystallization will occur. Mobile phase used in this HPLC method was not prepared with any salt-containing reagents, so it significantly reduces the phenomenon and possibility of crystallization. In addition, a longer life of the column and instrument is less costly, and fewer step in the procedure required for column cleansing can save time.

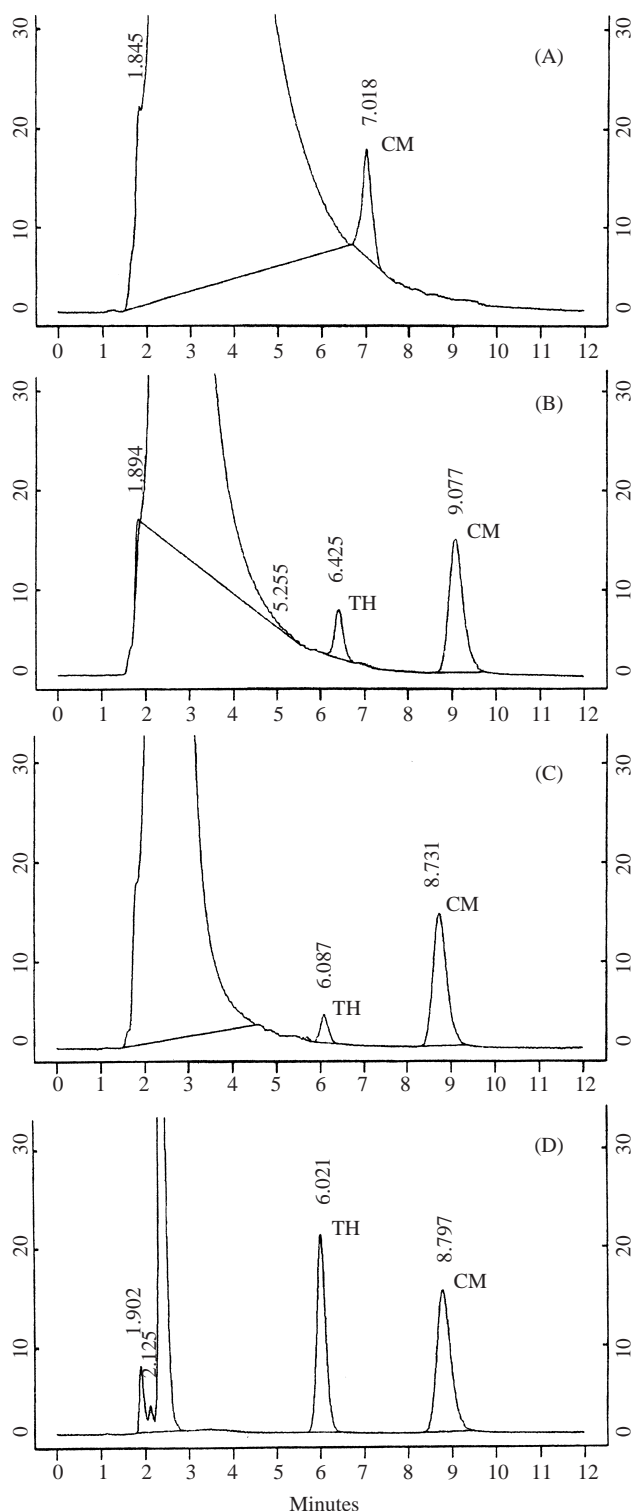


Figure 5. The effect of matrix pH change on retention time: (A) sample solution; (TH:25 $\mu\text{g/mL}$); (B) diluted sample solution (10 mL to 25 mL with mobile phase, TH:10 $\mu\text{g/mL}$) with internal standard (CM:8 $\mu\text{g/mL}$); (C) diluted sample solution (5 mL to 25 mL with mobile phase, TH:5 $\mu\text{g/mL}$) with internal standard (CM:8 $\mu\text{g/mL}$); (D) standard solution (TH:50 $\mu\text{g/mL}$) with internal standard (CM:8 $\mu\text{g/mL}$).

CONCLUSION

Compared to other traditional methods, the HPLC method utilizing silica column eluted with aqueous mixture containing trace amount of triethylamine and acetic acid provides a better alternative for the assay of Tetrahydrozoline HCl in ophthalmic solution that contains a comparatively high concentration of sulfamethoxazole sodium. Greater precision, accuracy, and peak-symmetry can be achieved. The impact of pH variation on retention time can be minimized by initially diluting the sample solution with the mobile phase. Additionally, this improved method demonstrated the potential to overcome the wide range of differences in the quantity of ingredients contained in pharmaceutical preparations by RP-HPLC assaying for TH. The key advantages of this method are reducing the for mobile phase preparation and cleaning column and instrument tubing, as well as improving of the ease of instrument maintenance. Normally, preparing the mobile phase, conducting the assay, and finishing the rinse of the column and instrument tubing are accomplished within 10 minutes.

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於矽膠層析管以含水性移動相分析定量眼藥水製劑中 Tetrahydrozoline Hydrochloride 成分

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摘 要

本研究利用高效液相層析法，以矽膠層析管分析，定量眼藥製劑中Tetrahydrozoline Hydrochloride成分。移動相為甲醇／水溶液（7:3, v/v）含Triethylamine 0.03% (v/v) 及Acetic acid 0.02% (v/v)，檢測波長為254 nm，流速1 mL/min，結果良好。回收率範圍為98.9 to 99.9%。在線性範圍為12.5 到 500 $\mu\text{g/mL}$ ($r^2 = 0.9996$) 和 1.0 到 20 $\mu\text{g/mL}$ ($r^2 = 0.9997$) 時均呈良好線性關係。本研究方法戡稱簡便迅捷，可解決以HPLC方法定量鹼性藥品常見Tailing及滯流時間長之缺點，並可有效節省移動相配製、層析管及儀器管道清洗時間，充分節省分析時間，同時由於移動相不添加任何鹽類，更能避免儀器因鹽析而阻塞或損害。

關鍵詞：tetrahydrozoline，眼用製劑，定量分析，高效液相層析法