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Original Article

A new and rapid HPLC method for the determination of phenoxyethanol in topical formulation

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ABSTRACT

Background and Aims: This study develops and validates a new liquid chromatographic method for determining the phenoxyethanol used as an antimicrobial preservative in pharmaceutic and cosmetic products. The study applies the developed high-performance liquid chromatography (HPLC) method to pomade formulation using a diode array detector to determine the phenoxyethanol.

Methods: The phenoxyethanol in the sample was analyzed in a C18 column (150 x 4.6 mm, 5 µm ID) under chromatographic conditions where the flow rate was determined as 1.0 mL/min. The column oven was 30.0°C, and phenoxyethanol was detected at 270 nm. Isocratic application of acetonitrile-water (50:50, v/v) was used as the mobile phase system. The validation of the developed method was performed according to the guidelines from the International Conference on Harmonisation (ICH, 2005) *Guidelines Q2 (R1).*

Results: The linearity range of the phenoxyethanol was 0.125-0.375 mg/mL, and the limits of detection and quantification were calculated as 31.25 ng/mL and 125.0 ng/mL, respectively. The assay recovery and precision of the phenoxyethanol from the pomade formulation were evaluated at 0.125 mg/mL, 0.250 mg/mL, and 0.375 mg/mL concentrations. The mean recoveries for phenoxyethanol in the pomade formulation were calculated at 99.99%-102.86%.

Conclusion: The validated method was successfully applied for determining phenoxyethanol in a topical formulation. The proposed method is cheap, fast, and simple and can be used safely for routine analysis.

Keywords: Phenoxyethanol, HPLC, DAD, cream formulation, validation, antimicrobial preservative

INTRODUCTION

Phenoxyethanol is one of the most popular preservatives used in many pharmaceutical and cosmetic products to protect against microbial growth (Figure 1). A large number of pharmaceutical and cosmetic preparations are known today to contain this preservative. Moreover, the products of some cosmetic brands are stated to be preserved with very high amounts of phenoxyethanol (Dreno et al., 2019).

Meanwhile, determining the amounts of active ingredients or excipients used for any reason, especially in pharmaceutical products, is an indispensable element of quality control. Although some studies have previously shown the wide use of phenoxyethanol to be safe, a lot of information is also found about its negative effects on human health (Dreno et al., 2019; Jakubczykz & Michalkiewicz, 2019). The amount of phenoxyethanol permitted in pharmaceutical preparations must be in the range of 0.5%-1% (Regulation of the European Community [EC], 2009). European Union Directive No. 1223/2009 states within its scope that the use of this substance in cosmetics should not exceed 1% (w/w; Agence nationale de sécurité du médicament et des produits de santé [ANSM], 2012). In fact, its use in baby products has been further restricted (ANSM, 2019).

When considering all these limitations, the ability to determine the amount of this substance in pharmaceutical or cosmetic products quickly, precisely, and accurately using validated methods becomes even more important. To date, researchers have reported many methods for the determination of phenoxyethanol alone or in combination with other preservatives in pharmaceutical formulations (Akhtar et al., 1996; Sharma et al., 2008; Shabir, 2010; Roy & Chakrabarty, 2013; Jakubczykz & Michalkiewicz, 2019; Algethami et al., 2023)

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and cosmetic products (Abad-Gil et al., 2021; Abad-Gil et al., 2022; Borremans et al., 2004; Jakubczykz & Michalkiewicz, 2019). Although most of these are based on the HPLC technique, studies on other methods are also found (Jakubczykz & Michalkiewicz, 2019; Algethami et al.,2023).

This study plans to develop an extremely rapid, easy, accurate, and precise method for analyzing phenoxyethanol when found alone in pharmaceutical preparations. After validating the developed method, the study applies it to the analysis of a topical cream containing phenoxyethanol. The proposed method is superior in terms of time and cost, as its analysis time is shorter than other methods. In addition, the rapid and ease of the sample preparation procedure in the topical formulation are one advantage the method has compared to other methods.

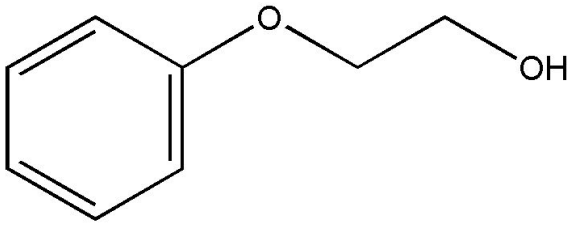

Figure 1. Chemical structure of phenoxyethanol.

MATERIAL AND METHODS

Chemicals

Phenoxyethanol (99%-100.5%) was provided by Brenntag Kimya. Ultrapure water from the Elga brand water system was used located in the research laboratory. Liquid chormatography (LC) grade acetonitrile was obtained from Merck (Germany). The pharmaceutical formulation was supplied by a pharmacy and Kurtsan İlaçları A.Ş.

Solutions

The phenoxyethanol stock solution (2.5 mg/mL) was dissolved in water. The linearity range for phenoxyethanol was prepared in the concentration ranges of 0.125, 0.187, 0.250, 0.312 and 0.375 mg/mL. The solutions were diluted using the mobile phase. Isocratic application of the acetonitrile-water mix (50:50, v/v) was used as the mobile phase system. This was then sonicated for 20 min.

HPLC System

The study uses a liquid chromatographic system equipped with an autosampler, a column oven compartment, and a diode-array detector (DAD). The device used in the analysis is the Shimadzu LC 20A system (Kyoto, Japan). Separation was performed with a C18 column (150 x 4,6 mm, 5 µm ID) under chromatographic conditions where the flow rate was determined as 1.0 mL/min. The column oven was 30.0°C, and phenoxyethanol was detected at 270 nm. The chromatographic data, analysis, and reporting were performed via the LC-Solution system software.

Preparing the sample solutions

The sample solution was prepared in mobile phase. The cream formulation is dissolved in the acetonitrile-water mix (50:50, v/v) to obtain a concentration of 0.25 mg/mL. Next, the sample solution is stirred in a vortex mixer for 60.0 s and then kept in an ultrasonic bath for 10 min. The mixture is then filtered with a 0.45 µm nylon filter and injected into the HPLC system. The amount of substance in the cream formulation is calculated by substituting the resulting area into the calibration equation.

Validation

The developed method has been validated using the guidelines from the International Conference on Harmonisation (ICH, 2005) *Text and Methodology Q2 (R1)*. The linearity of the phenoxyethanol is studied in the range of 0.125-0.375 mg/mL by taking into account the phenoxyethanol concentration in the preparation containing the solution for injection. The limits of detection (LOD) and limits of quantitation (LOQ) are calculated using the signal-to-noise ratio formula. Absolute recoveries are evaluated using the placebo addition by selecting the initial, middle, and final concentrations of the calibration curve. A placebo solution is prepared similar to the sample solution. Three different concentrations of standard phenoxyethanol solutions (0.125, 0.250, and 0.375 mg/mL) were filled to the same volume using the placebo solution, and these mixtures have also been analyzed using the recommended method.

Precision studies of the method were examined with inter-day and intra-day precision. Therefore, separate standard solutions were prepared at concentrations of 0.125, 0.250 and 0.375 mg/mL in the mobile phase solution. Standards from the same day and different days were analyzed and assessed by calculating the relative standard deviation percentages (%RSDs) of the field values.

The stability of phenoxyethanol in the cream formulation solution was studied at the end of the 12^{th} and 24^{th} hours of the samples being kept under autosampler conditions. The stability was assessed by comparing the initial results with those obtained upon the conclusion of the analyses. The robustness parameter was assessed by changing the mobile phase flow rate and column temperature. The standard solution was initially injected using a method flow rate of 1.0 mL/min. Subsequently, injections were conducted after adjusting the system to flow rates of 0.9 mL/min and 1.1 mL/min. A similar approach was taken for column temperature, with initial injections made at the method-specified temperature of 30.0°C. The standard solution was injected by adjusting the column temperature first to 28°C

and then to 32°C. The obtained chromatograms were analyzed for the theoretical plate numbers and tailing factors, which are the essential parameters for evaluating system suitability.

RESULTS AND DISCUSSION

The developed and validated HPLC method involves a simple sample preparation and is a selective, reproducible, and reliable method enabling the analysis of phenoxyethanol from a cream formulation based on HPLC using DAD.

Selectivity

To assess the method selectivity, the system received injections of the mobile phase, phenoxyethanol standard solution, cream formulation, and placebo solutions. During the retention time of phenoxyethanol, no peaks were observed due to the solvent or placebo, as depicted in Figure 2.

Linearity and sensitivity

Linearity was studied in the proposed method in the concentration range of 0.125-0.375 mg/mL by taking the analyzed phenoxyethanol formulation into consideration. The average regression formula can be expressed as:

$$A = 10472711C + 91526 (r = 0.9997) \quad (1)$$

where C represents the concentration of phenoxyethanol (mg/mL) and A represents the peak area. The results for linearity from the proposed method are displayed in Table 1. In accordance with the study parameters, LOD and LOQ results were determined as 31.25 ng/mL and 125.0 ng/mL, respectively.

Table 1. Linearity results obtained from the developed method

Parameter	Phenoxyethanol
Linearity range (mg mL^{-1})	0.125-0.375
Regression equation	A = 10472711C + 91526
Slope ± SD	10472711± 2755
Intercept ± SD	91526± 762
Mean correlation coefficient, r	0.9997
LOD[a] (ng mL^{-1})	31.25
LOQ[b] (ng mL^{-1})	125.0

[a] Limits of Detection; [b] Limits of Quantitation

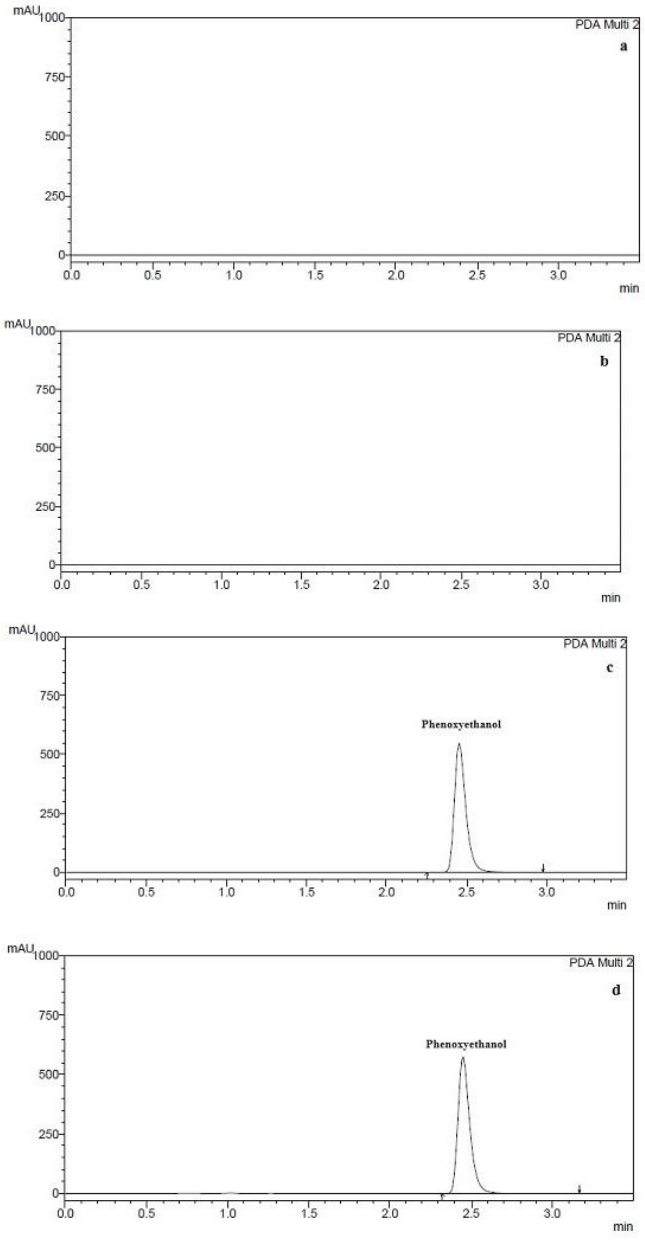

Figure 2. The chromatograms obtained from (a) the mobile phase, (b) the placebo solution, (c) the standard phenoxyethanol solution (0.250 mg/mL), and (d) the cream formulation (1.0 g).

Recovery

As shown in Table 2, the absolute recovery values of phenoxyethanol in the cream formulation were found between 99.99-102.86%. The average phenoxyethanol recovery was calculated as 101.07%.

Table 2. Recovery results for the phenoxyethanol assay.

Concentration (mg mL^{-1})		Recovery (%)	RSD[b] (%)
Added	Found (mean ± SD[a])		
0.125	0.129±0.000$_1$	102.86	0.039
0.250	0.251±0.000$_1$	100.36	0.026
0.375	0.375±0.000$_1$	99.99	0.007

[a] Standard deviation; [b] Relative standart deviation

Precision

Precision assessments were obtained with both intra-day and inter-day repeatability, as outlined earlier. The %RSD ranged from 0.008%-0.442% for intra-day repeatability and from 0.010%-0.448% for inter-day repeatability. Table 3 displays the precision values for the method. These results are in accordance with the statement that the %RSD value should be less than 2.0%.

Table 3. Intra-day & inter-day precision and accuracy of phenoxyethanol (n = 6)

Concentration (mg/mL)		RSD[b] (%)	RME[c] (%)
Added	Found (mean ± SD[a])		
Intra-day			
0.125	0.123 ± 0.000$_0$	0.008	-1.441
0.250	0.250 ± 0.001	0.442	0.123
0.375	0.375 ± 0.000$_0$	0.008	-0.012
Inter-day			
0.125	0.123 ± 0.000$_1$	0.069	-1.489
0.250	0.251 ± 0.001	0.448	0.407
0.375	0.375 ± 0.000$_0$	0.010	-0.004

[a] Standard deviation; [b] Relative standart deviation; [c] Relative mean error

Stability

Assessing the stability of the proposed method was computed under the conditions determined for the phenoxyethanol solution by comparing the initial results with those obtained at the conclusion of the analyses after 12 and 24 hours had passed. Upon analyzing the obtained values, the variations were seen to range between 0.50‰-0.39‰ (Table 4). These results show no noticeable alteration to have occurred in the peak areas.

Table 4. Stability results for the phenoxyethanol obtained using the proposed method

Time (hour)	Concentration (mg/mL) (mean±SD)	RSD (%)	Variation (%)
0	0.273±0.000$_1$	0.029	0.00
12	0.274±0.000$_1$	0.023	0.50
24	0.274±0.000$_1$	0.022	0.39

Robustness

The robustness of the method was tested by evaluating the results obtained from changing the flow rate and column oven temperature. The average tailing factor using the proposed method was determined as 1.530 ± 0.007 at a flow rate of 1.0 mL/min and a column temperature of 30.0°C. While assessing the robustness of the method, adjustments were made to the column temperature and mobile phase, as previously outlined. The resulting values for the tailing factor were within the range of 1.531-1.540 and 1.492-1.537, respectively. Additionally, the theoretical plate number, which was initially determined to be 29,816 ± 896 using the proposed method, varied between 27,433-31,337 for the mobile phase changes and between 28,438-29,252 for the column temperature changes. The method was determined to have remained unaffected by minor changes.

Determination of phenoxyethanol from the topical formulation

The percentage of phenoxyethanol in the cream formulation as determined by the proposed method was calculated in the range of 98.65%-98.90% (Table 5). These outcomes align with the specified range of 95.0% to 105.0% as outlined in ICH's (2005) *Text and Methodology Q2* (R1). This proves the applied method to have been successful at analyzing the phenoxyethanol within the cream preparation.

CONCLUSION

This study has developed an extremely rapid, easy, and accurate method for analyzing phenoxyethanol in pharmaceutical prepa-

Table 5. Determination of the phenoxyethanol in a topical formulation (n = 6)

n	g / 100 g	%
1	0.9868	98.68
2	0.9869	98.69
3	0.9865	98.65
4	0.9890	98.90
5	0.9885	98.85
6	0.9887	98.87
Mean	0.9877	
SD[a]	0.001	
RSD[b]	0.115	

[a] Standard deviation; [b] Relative standart deviation

rations. After validating the developed method in accordance with ICH rules, the method was then successfully applied to the analysis of a topical cream containing phenoxyethanol.

Compared to other HPLC techniques, the developed method is shorter in terms of analysis time (Akhtar et al., 1996; Sharma et al., 2008; Sabir, 2010; Roy & Chakrabarty, 2013; Algethami et al., 2023; Abad-Gil et al., 2021; Abad-Gil et al., 2022; Borremans et al., 2004) using a standard HPLC instrument, with the analysis time being completed in 3.5 minutes. This is very useful in terms of time and costs for pharmaceutical companies' routine analyses. In addition, the sample preparation of the developed method includes the injection into the device after a 10-minute degassing of the mobile phase. This is an extremely simple procedure for topical formulations that might otherwise require quite a challenging sample preparation.

As a result, the developed method is extremely rapid and simple, in addition to being highly accurate and precise, and will bring advantages to the routine analysis of phenoxyethanol in topical formulations, especially in terms of time and cost.

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