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Research Article

Simultaneous Estimation of Four Preservatives in Pharmaceutical Ointment by RP-HPLC

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ABSTRACT

A simple, specific, precise and accurate reverse phase HPLC method has been developed for the simultaneous determination of preservatives (Methyl Para hydroxybenzoate, Ethyl para hydroxybenzoate, Propyl para hydroxybenzoate and Chlorocresol) in ointment. The chromatographic separation was achieved on Zorbax column, 150 mm × 4.6 mm, 5 µm, using PDA detector. The mobile phase was 50% Methanol (v:v), at a flow rate of 1.0 mL/min. All preservatives were detected at 230 nm, at 30°C temperature for column. The retention times were 2.15 min for Methyl para hydroxybenzoate, 2.70 min for Ethyl para hydroxybenzoate, 3.69 min for Propyl para hydroxybenzoate and 4.01 min for Chlorocresol, with incredibly good resolution between peaks. The method was validated according to the ICH guidelines with respect to specificity, linearity (r2 =0.999; 0.998; 0.999 and 0.998), accuracy (99 to 100.1%), precision (RSD<2%).

Keywords: Preservatives; Ointment; Simultaneous determination; RP- HPLC; Validation

INTRODUCTION

In the 21st century, accelerated lifestyle has greatly changed people's diet. In order to extend the expiry date of canned food, at the expense of quality, certain types of preservatives are being used. The preservatives help maintain the formula and sustainability of the product. The use of preservatives in food industry is a well-known fact, but they are also used in cosmetic and pharmaceutical industries [1]. Ideally, preservatives should meet the following conditions: possess a wide range of antimicrobial activity that includes Gram-positive and Gram-negative bacteria and fungi, be chemically and physically stable during the shelf life of the product, and not be toxic [2]. Many preservatives are available for use in pharmaceutical preparations (benzoic acids and salts, sorbic acids etc.) [3]. Parabens are a series of para hydroxybenzoates or esters of para hydroxybenzoic acid. They are known preservatives and are used as bactericides and fungicides (Methyl para hydroxybenzoate, Ethyl para hydroxybenzoate, Propyl para hydroxybenzoate and Chlorocresol) [4]. British Pharmacopoeia (BP) [5] and United States Pharmacopoeia (USP) [6] do not describe HPLC method for its estimation in pharmaceutical ointment. The study describes simple, sensitive, accurate, precise and rapid RP-HPLC method for determination of four preservatives.

MATERIALS AND METHODS

Instrumentation

HPLC system (Agilent technology) consisting of gradient pump, Auto sampler, column oven and photodiode array detector (PDA, Agilent technology) was employed for analysis. Chromatographic data was acquired using ChemStation software.

Reagents and materials

Methyl para hydroxybenzoate, Ethyl para hydroxybenzoate, Propyl para hydroxybenzoate and Chlorocresol were supplied by USP. Methanol, Ethanol 96% and Acetonitrile (HPLC, Semikem), Milli-Q Water.

Chromatographic condition

Zorbax column, 150 mm × 4.6 mm, 5 µm was used as a stationary phase. The mobile phase consists of Methanol and Milli-Q Water in the ratio of 50:50 (v/v). The flow rate of the mobile phase was 1.0 mL/min. Detector signal was monitored at a wavelength of 230 nm. The column temperature was kept at 30°C and injection volume was 20 µL.

Preparation of standard solutions

The standard stock solution was prepared by transferring 7.5 mg each of preservatives in a 20 mL volumetric flask. Then each solution was diluted to obtain final standard concentration of Methyl para hydroxybenzoate, Ethyl para hydroxybenzoate, Propyl para hydroxybenzoate and Chlorocresol from 0.8 mg/L to 12 mg/L, respectively.

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Preparation of sample solutions

About 0.75 g of ointment was weighed into a tared Erlenmeyer flask, 50 mL of 96% Ethanol was added, and heated gently on a bath at 50°C to dissolve the fat completely. It is then cooled under a stream of cold water, creating a greasy ball again. Of these solutions, 5 mL were pipetted and supplemented with 50% Acetonitrile to 50 mL.

Ointment sample solution with added preservatives

Four sample solutions were made by spiking solutions of standard and a sample at a concentration of 0.8 mg/L.

Validation of the Proposed Method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [7].

System suitability: The system suitability test as per method was performed and checked before performing any parameter (%RSD).

Linearity and range: A standard linearity solution was prepared to different concentration of 50%, 80%, 100%, 120%, and 150% of the target concentration.

Method precision (Repeatability): Six solutions were prepared from Methyl para hydroxybenzoate, Ethyl para hydroxybenzoate, Propyl para hydroxybenzoate and Chlorocresol. Then they were injected by one analyst and analyzed on same day.

Accuracy: The accuracy of the method was carried out at three levels in the range of 80-120% of the working concentration of the sample. Calculated amount of Methyl para hydroxybenzoate, Ethyl para hydroxybenzoate, Propyl para hydroxybenzoate and Chlorocresol working standards were added in placebo containing volumetric flasks to prepare 80%, 100% and 120% level of the working concentration.

Specificity: A blank preparation, standard preparation and placebo preparation were prepared and injected.

RESULTS AND DISCUSSION

The method has provided adequate separation for Methyl para hydroxybenzoate, Ethyl para hydroxybenzoate, Propyl para hydroxybenzoate and Chlorocresol pharmaceutical ointment.

Specificity of the chromatograms were checked for the appearance of any extra peaks. No chromatographic interference from ointment excipients was found.

Linearity of preservatives was in the range of 0.8-12 mg/L. The data for the peak area against the concentration were treated by linear regression analysis and the correlation coefficient value obtained were 0.999, 0.998, 0.999 and 0.998.

Separation was obtained by using Zorbax column, 150 mm × 4.6 mm, 5 µm as a stationary phase at 30°C temperature and using a mobile phase 50% Methanol at a flow rate 1.0 mL/min and wavelength for detection was 230 nm. Under these optimized conditions, the analyte peaks were well resolved and were free from tailing. The tailing factors were less than 1.5 for all preservatives. The elution orders were 2.15 min for Methyl para hydroxybenzoate, 2.70 min for Ethyl para hydroxybenzoate, 3.69 for Propyl para hydroxybenzoate and 4.01 for Chlorocresol at a flow rate of 1.0 mL/min. Specificity of chromatograms are shown in Figure 1 and system suitability was established by injected standard solution and the results are shown in Table 1

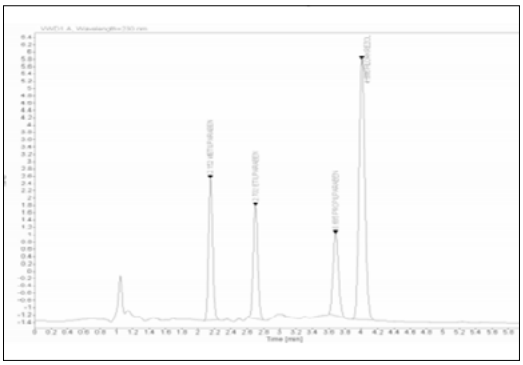

Figure 1: Chromatogram of well-separated preservative peaks.

Component	Area	Symmetry	Plate	Resolution
Methyl para hydroxybenzoate	13.63	0.89	8796	-
Ethyl para hydroxybenzoate	11.95	0.89	11232	5.69
Propyl para hydroxybenzoate	10.52	0.88	14247	8.74
Chlorocresol	35.35	0.90	15452	2.55

Table 1: System suitability parameters.

The accuracy was expressed as the percentage of analytes recovered by the assay method. It was confirmed from results that the method is accurate (Table 2).

Level (%	RSD				Mean RSD			
	Methyl para hydroxybenzoate	Ethyl para hydroxybenzoate	Propyl para hydroxybenzoate	Chlorocresol	Methyl para hydroxybenzoate	Ethyl para hydroxybenzoate	Propyl para hydroxybenzoate	Chlorocresol
80	100.6	95.3	98.5	95.2	100.1	100.1	99.2	99
100	99.9	101.1	100.4	101.9				
120	100.2	100.5	101	99.8				

Table 2: Accuracy data.

In Precision, the relative standard deviations (RSD) were 0.4% for Methyl para hydroxybenzoate, 0.7% for Ethyl para hydroxybenzoate, 1.1% for Propyl para hydroxybenzoate and 1.1% for Chlorocresol, which are well within the acceptable limit of 2.0%.

Results for four spiked samples are expressed as recovery percentage and presented in Table 3.

Sample	Methyl para hydroxybenzoate	Ethyl para hydroxybenzoate	Propyl para hydroxybenzoate	Chlorocresol
	Recovery (%)	Recovery (%)	Recovery (%)	Recovery (%)
Sample 1	98.07	101.83	102.55	101.51
Sample 2	98.64	103.67	97.45	101.51
Sample 3	104.14	104.08	97.82	101.80
Sample 4	98.79	103.75	97.91	101.17

Table 3: Spike recovery data for preservatives.

CONCLUSION

Proposed RP-HPLC method is specific, accurate and precise for the simultaneous determination of Methyl para hydroxybenzoate, Ethyl para hydroxybenzoate, Propyl para hydroxybenzoate and Chlorocresol from pharmaceutical ointment. The newly developed method is simple and cost effective as it uses simple mobile phase. The separation for represented preservatives was attained in just six minutes. The method was validated as per ICH guidelines. All other parameters such as specificity, linearity, precision and accuracy, passes the criteria set forth by ICH guidelines. The described method is suitable for routine analysis and quality control either of pharmaceutical preparations containing these preservatives as such or in combination.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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