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High Performance Liquid Chromatographic Determination of Sodium Benzoate, Methylparaben and Propylparaben as Preservative Components in Nystatin Suspensions

A. Abdollahpour[a], M. Forouhi[b], M. Shamsipur[c] and Y. Yamini[a,*]
[a]*Department of Chemistry, Tarbiat Modares University, Tehran, Iran*
[b]*Jaber Ebne Hayyan Pharmaceutical Industry, Tehran, Iran*
[c]*Department of Chemistry, Razi University, Kermanshah, Iran*

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A simple and sensitive method was developed for the analysis of preservatives sodium benzoate, methylparaben and propylparaben in nystatin suspensions by reversed-phase high performance liquid chromatography (HPLC), equipped with a C18 column and PDA detector. The mobile phase was a mixture of acetonitrile and acetate buffer of pH 4.4 (35:65 v/v). Under the optimized experimental conditions, separation of the preservatives was achieved in less than 20 min. The limits of quantifications (LOQs) and the linear dynamic ranges (LDRs) of sodium benzoate, methylparaben and propylparaben were 0.3 and 50-1000 μg ml^{-1}, 0.5 and 50-600 μg ml^{-1} and 0.3 and 50-900 μg ml^{-1}, respectively; the respective precisions (%RSD) at 500 μg ml^{-1} level were 0.72%, 0.73% and 0.51% (n = 6). The average recoveries of sodium benzoate, methylparaben and propylparaben for spiked nystatin samples were obtained as 98%, 97% and 98%, respectively.

Keywords: Preservative, Determination, Nystatin, Reversed phase, HPLC

INTRODUCTION

The macrolide antibiotic nystatin (NYS) was first isolated from the actinomycete species *Streptomyces noursei* in 1950 and launched into antifungal therapy only five years later [1]. Nystatin exerts both a fungistatic and fungicidal action against *Candida Albicans*. For the treatment of oral candidiasis, this drug is administrated in either a suspension or gel dosage form (100,000 IU) taken 4-5 times daily [2].

In most pharmaceutical preparations, especially in syrups, preservation is essential because the excipients, and sometimes the drug itself, may be destroyed by different micro-organisms and consequently the formulation breaks down. Sodium benzoate (SB) and alkyl esters of *p*-hydroxybenzoic acid (parabens) including methylparaben (MP) and propylparaben (PP) are usually used as preservatives to prevent foods, cosmetics, and pharmaceuticals from microbial and fungal attacks [3-7]. Their toxicity is generally low owing to fast hydrolysis *in vivo* to parent acid, which is rapidly conjugated and excreted [8]. The antimicrobial activity of parabens increases with increasing length of the alkyl chain of ester group but, in practice, shorter esters are commonly used because of their high solubility in water [9].

Sodium benzoate is used at concentrations of 0.02-0.5% in oral medicines, 0.5% in parenteral products, and 0.1-0.5% in cosmetics. Propylparaben 0.02% together with methylparaben 0.18% has been used for the preservation of various parenteral pharmaceutical formulations. A mixture of parabens is thus

*Corresponding author. E-mail: yyamini@modares.ac.ir

frequently used to provide effective preservation [10]. Recently, preservatives in consumer products have received close attention because of their possible side-effects on humans. The HPLC analysis of methylparaben and propylparaben is frequently described in the literature [11-13]. However, many of the reported methods use complicated and labor-intensive pre-treatment procedures. The USP 30 test for sodium benzoate, methylparaben, and propylparaben is the only assay determination for each preservative in the raw material and there is no report for the determination of these compounds in the finished products such as nystatin [14].

In the present work we report a simple procedure for extraction and HPLC separation and determination of a mixture of sodium benzoate, methylparaben and propylparaben. The method in question was applied to the analysis of the preservatives in the commercial drug samples.

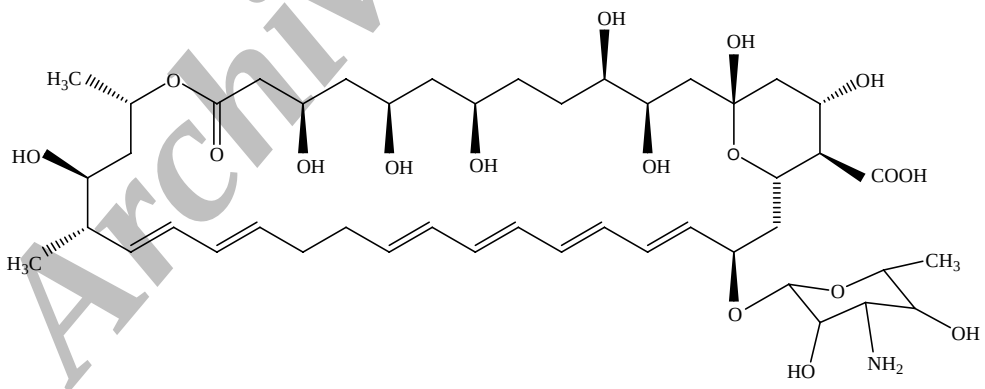
EXPEIMENTAL

Chemicals

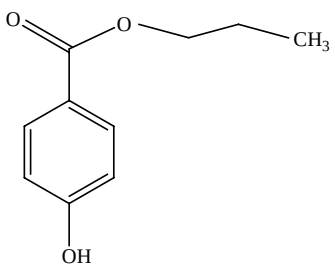
Sodium benzoate (SB), methylparaben (MP) and propylparaben (PP), ammonium acetate and acetic acid were analytical grade and obtained from Merck (Darmstadt, Germany). HPLC grade methanol and acetonitrile were also purchased from Merck (Darmstadt, Germany). All of the solvents were filtered and degassed before use. Nystatin suspension samples were obtained from a commercial source (Jaber Ebne Hayyan Pharmaceutical Industry, Iran). Double-distilled and deionized water was filtered through active charcoal and a 0.5 µm filter and used in mobile phase.

Chromatographic System

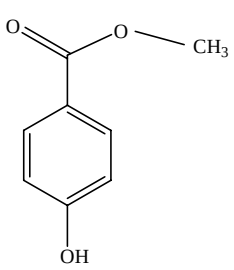
The HPLC instrument used consisted of a computer-controlled system with chromgate software and Knauer low pressure HPLC pump K-1001, Knauer solvent organizer K-1500, and PDA detector K-2800 operated at 254 nm for quantitative analysis. The column was a Chromolit C18 (25 cm × 4.6 mm, 5.0 µm). The flow rate of mobile phase in all separations was optimized at 1 ml min^{-1} and the sample injection volume was 20 µl. The injection of the samples was carried out using a Rheodyne model 7725i manual injector. The experiments were performed at ambient temperature and



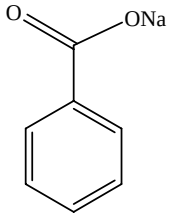
NYS



PP



MP



SB

under isocratic elution conditions using a mixture of acetonitrile and an aqueous acetate buffer of pH 4.4 (35:65 v/v).

Sample Preparations

1000 µg ml^{-1} stock standard solutions of the preservatives were prepared by dissolving a proper amount of the compounds in methanol. Working standard solutions were prepared by the proper dilution of the stock solutions in methanol.

1.0000 g of the nystatin suspension powder was accurately weighed and transferred into a test tube. Twenty-five milliliters of methanol were added and the mixture was placed in a water bath at 50 °C for 30 min. The content was filtered and the clear filtrate was injected directly into the chromatographic system.

RESULTS AND DISCUSSION

To optimize the chromatographic separation conditions, various mixtures of acetonitrile and acetate buffer of pH 4.4 were used. The results showed that an increase in the amount of acetonitrile in the solvent mixture decreases the analytes retention times. A binary mixture with an acetonitrile/acetate buffer ratio of 35:65 v/v was then selected as the best practical mobile phase. The flow rate of mobile phase was optimized at 1 ml min^{-1}. The experiments were performed at ambient temperature and under isocratic elution conditions. The chromatogram of a standard solution containing 100 µg ml^{-1} of SB, MP and PP is shown in Fig. 1. As is obvious, Fig. 1 shows an ideal separation of the three preservatives at a time period of about 16 min.

The stability of the standard solutions of SB, MP and PP was monitored by measuring the area of response of 20 µl injections over a period of 7 days. As mentioned in Handbook of Pharmaceutical Excipients [10], over a pH range of 3-6 and at room temperature, all the corresponding aqueous solutions were found to be stable (less than 10% decomposition) for up to about 4 days [11]. The linearity of SB, MP and PP in standard solutions was investigated at 6 different

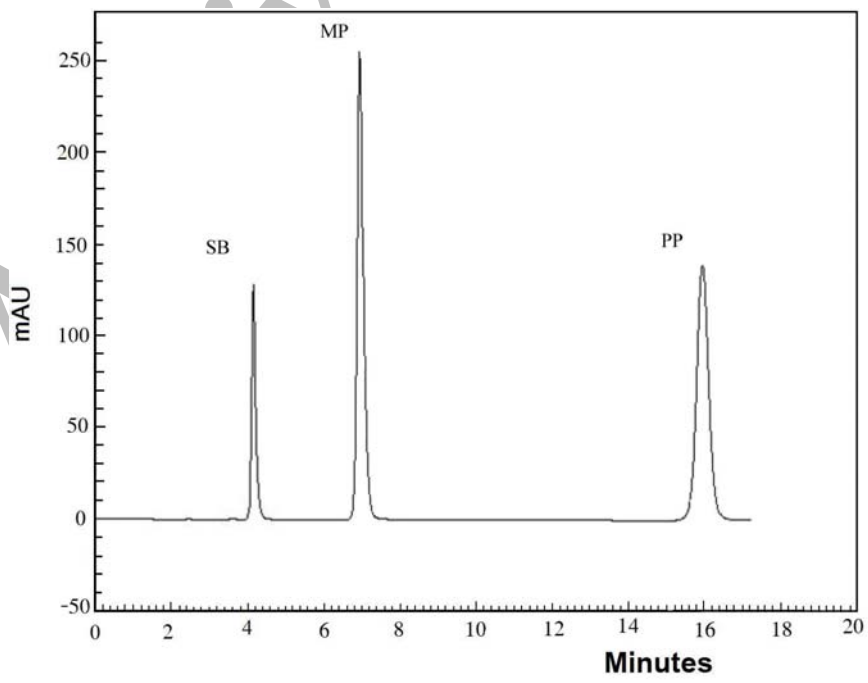


Fig. 1. Chromatogram of standard solution of SB, MP and PP. Mobile phase: 35:65 (v/v) acetonitrile-acetate buffer (pH 4.4), column: C18 and detection at 254 nm.

concentration levels. The resulting calibration curves for these solutions were linear in the range of 50-1000 μg ml^{-1} for SB, 50-600 μg ml^{-1} for MP, and 50-900 μg ml^{-1} for PP, with the respective regression coefficients (for five replicates at each concentration level) of 0.999, 0.995 and 0.998. Table 1 summarizes the calibration curve equations, relative standard deviation (%RSD), regressiuon coefficient (R^2), linear dynamic range (LDR), limit of detection (LOD) and limit of quantitation (LOQ) values obtained by the proposed method for SB, MP and PP.

The proposed procedure was validated by means of recovery experiments. Two solutions of SB, MP and PP as standard samples at different concentration levels (*i.e.*, 50 and 500 μg ml^{-1}) were prepared in methanol. Then a drug-free sample was spiked with known amounts of SB, MP and PP at the two concentration levels. The recoveries were estimated by the comparison of the obtained results from the spiked samples with those from direct injection of standard samples, under the same experimental conditions. As is obvious from Table 2, the average recoveries for SB, MP and PP from the spiked samples are 98%, 97% and 98%, respectively.

The developed method is simple, precise and rapid. A typical HPLC chromatogram of a real drug sample is shown in Fig. 2. The analysis of different nistatin samples revealed that the three preservatives are present in their actual concentrations according to their declared formulations.

CONCLUSIONS

Determination of preservatives SB, MP and PP has been performed by reversed-phase HPLC in a single step. This method offers the following advantages: (1) higher selectivity for SB, MP and PP, (2) fewer interfering peaks and cleaner baseline and (3) shorter analysis time. The results obtained were precise and accurate and the limits of detection of preservatives were sufficiently low. The method can be used for the routine analysis (batch analysis) of compounds in pharmaceutical products containing preservatives. The proposed method was successfully applied to the identification and quantitative analysis of the preservatives in nystatin suspensions.

Table 1. Calibration Results for Preservatives SB, MP and PP

Preservative	Equation	%RSD) (n = 5)	R^2	LDR (μg ml^{-1})	LOD (μg ml^{-1})	LOQ (μg ml^{-1})
SB	y = 7440.2 C (μg ml^{-1}) + 6.4×10^3	0.72	0.999	50-1000	0.1	0.3
MP	y = 19183 C (μg ml^{-1}) + 5.0×10^7	0.73	0.995	50-600	0.1	0.5
PP	y = 113402 C (μg ml^{-1}) + 2.0×10^6	0.51	0.998	50-900	0.1	0.3

Table 2. Recoveries and Coefficient of Variation for SB, MP and PP, Based on 3 Replicate Measurements, from Spiked Sample at Different Levels

	SB		MP		PP	
Spiked	50 μg ml^{-1}	500 μg ml^{-1}	50 μg ml^{-1}	500 μg ml^{-1}	50 μg ml^{-1}	500 μg ml^{-1}
Recovery	94%	98%	95%	97%	93%	98%
RSD (%)	2	3	3	4	3	2

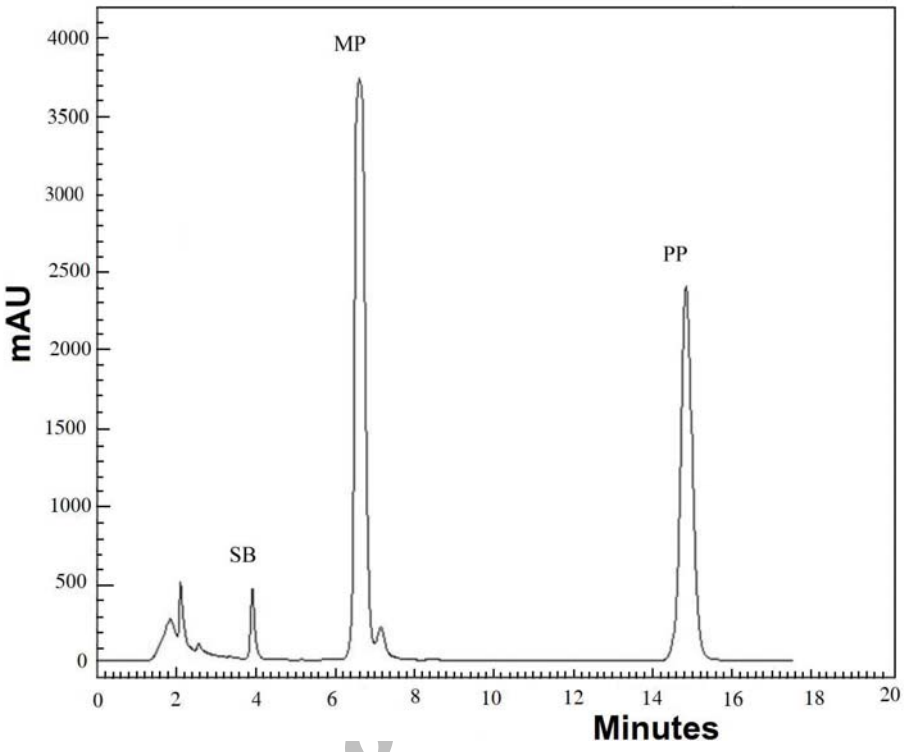


Fig. 2. Chromatogram of SB, MP and PP in a real sample. Mobile phase: 35:65 (v/v) acetonitrile-acetate buffer (pH 4.4), column: C18 and detection at 254 nm.

REFERENCES

[1] E.L. Hazen, R. Brown, Science 423 (1950) 112.

[2] USP DI 2004, 24th ed., Drug Information for the Health Care Professional (2004).

[3] F.D. Croo, J.D. Schutter, W. Van den Bossche, P.D. Moerloose, Chromatographia 260 (1984) 18.

[4] H.J. Lin, Y.M. Choong, J. Food Drug. Anal. 291 (1999) 7.

[5] Y.J. Heo, K.J. Lee, J. Pharm. Biomed. Anal. 1371 (1998) 17.

[6] S.P. Wang, C.L. Chang, Anal. Chim. Acta 85 (1998) 377.

[7] Y.H. Lin, S.S. Chou, F. Sheu, Y.T. Shgu, J. Chromatogr. Sci. 345 (2000) 38.

[8] D. Ivanovic, M. Medenica, E.N. Guernet, M. Guernet, Chromatographia 652 (1995) 40.

[9] G. Burini, J. Chromatogr. A 213 (1994) 664.

[10] A.H. Kibbe, Handbook of Pharmaceutical Excipients, 3th ed., 2000, pp. 340, 450, 471.

[11] R. Hajkova, P. Solich, M. Pospisilova, J. Sicha, Anal. Chim. Acta 91 (2002) 467.

[12] J.A. Arancibia, M.A. Boldrini, G.M. Escandar, Talanta 261 (2000) 52.

[13] The European Pharmacopoeia, 4th ed. (Suppl. 4.3), Council of Europe, Strasbourg, France, 2003, p. 3065.

[14] USP 30, United States Pharmacopoeial Convention Inc., 2007, p. 136.