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Journal of Pharmaceutical and Biomedical Analysis 38 (2005) 763–767
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

A novel gradient HPLC method for simultaneous determination of ranitidine, methylparaben and propylparaben in oral liquid pharmaceutical formulation

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Received 4 September 2004; received in revised form 8 February 2005; accepted 8 February 2005
Available online 23 March 2005

Abstract

A selective and accurate high-performance liquid chromatographic method has been developed and validated for the simultaneous determination of ranitidine, methylparaben (MP) and propylparaben (PP) in oral liquids. Samples were purified by solid-phase extraction (SPE) using a copolymeric [poly(divinylbenzene-co-*N*-vinylpyrrolidone)] sorbent. The chromatographic separation was achieved by HPLC using a mixture of ammonium acetate solution (0.5 M), acetonitrile and methanol as the mobile phase with gradient elution, a Nucleosil C18 column and UV detection at 254 nm. The method was validated with respect to linearity, precision, accuracy, selectivity, and robustness. All the parameters examined met the current recommendations for bioanalytical method validation. The method was found to be applicable to routine analysis (assays and stability tests) of active compound (ranitidine) and preservatives (MP and PP).

Keywords: Parabens; Ranitidine; Reversed phase HPLC; Solid-phase extraction; Oral pharmaceutical formulation

1. Introduction

Ranitidine is a H_2-receptor antagonist commonly used in the treatment of duodenal and gastric ulceration [1]. Ranitidine can be found in many pharmaceutical forms such as tablets, injectable solutions and oral liquids. Compared to the oral liquid formulation, the tablets are preferably used. In addition, oral liquids require the presence of antimicrobial agents such as parabens. However, in the case of infants, children or geriatric patients the liquid form should be used rather than solid dosage form, as it is easier and safer to swallow [2]. Apart from that, liquid formulations favor a most rapid absorption of the active substance rather than solid forms.

Parabens, a group of alkyl esters of *p*-hydroxybenzoic acid (PHBA) are widely used as antimicrobial preservatives in cosmetics, food and pharmaceutical products [3]. The parabens are effective over a wide pH range and present a broad spectrum of antimicrobial activity, although they are most effective against yeast and molds. Methylparaben (0.18%) and propylparaben (0.02%) have been used for the preservation of various parenteral pharmaceutical formulations.

Parabens degrade by hydrolysis under alkaline and acidic conditions to form *p*-hydroxybenzoic acid, which shows little preservative action. When oral pharmaceutical liquid containing MP, PP, ranitidine and several excipients (polyols such as sorbitol or glycerol) was analyzed by reversed-phase HPLC, several unknown polar peaks were observed near the solvent front. These peaks could arise from degradation products due to an interaction between parabens or PHBA and sorbitol [4,5]. It seems clear that pharmaceutical formulations containing polyols and parabens would present different chromatographic profiles after storage, owing to the formation of these degradation products. Due to the presence of such unknown peaks, a clean-up procedure was accomplished using solid-phase extraction (SPE).

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doi:10.1016/j.jpba.2005.02.022

A great number of assay methods for determination of ranitidine in pharmaceutical formulations and biological fluids has been reported using C18 [6–9], Lichrospher 60 RP-select B [10] and phenyl [11] columns. For the determination of parabens in food, cosmetics and pharmaceutical formulations, the most commonly used analytical column is C18 [12–20]. The use of cyano column has also been reported [5,21]. Some of these methods describe a SPE clean-up procedure prior to HPLC. As far as ranitidine is concerned, the SPE clean-up methods reported refer to the determination of the drug substance in human plasma. On the other hand, parabens have been extracted from matrices similar to pharmaceutical oral liquids using C18 [15] and Oasis HLB (Waters) cartridges [14].

To the best of our knowledge there are no data describing the use of HPLC for the simultaneous determination of ranitidine, MP and PP.

This paper describes a precise, simple and reliable HPLC method with gradient elution and UV detection for the simultaneous determination of the active compound (ranitidine) and preservatives (MP and PP) after SPE clean-up procedure. The method has been proved to be suitable for bulk, final product release and stability testing in liquid pharmaceutical formulations.

2. Experimental

2.1. Materials

Methyl 4-hydroxybenzoate (purity 99.0%) and propyl 4-hydroxybenzoate (purity 99.0%) were purchased from Neochema. Ranitidine HCl BPCRS (purity 89.4% as ranitidine) was purchased from British Pharmacopoeia. Deionised distilled water was used throughout the experiments. Acetonitrile and methanol from J.T. Baker were HPLC grade and ammonium acetate from Panreac was analytical grade. Cartridges for SPE (Oasis HLB 3 cc, 60 mg) were supplied by Waters.

2.2. Equipment/chromatographic system

HPLC analysis was performed on a Shimadzu LC 2010 C system, equipped with a model series SPD-M10A detector, a gradient elution pump with degassing device and mixer, a cooling autosampler and a column heater/cooler. The diode array detector was used for the spectrum extraction while the analysis was carried out at 254 nm. The separation was achieved using a Nucleosil 100-5 C18 250 mm × 4.6 mm, 5 μm (Macherey–Nagel) stationary phase. The data was acquired via Class VP data acquisition software, Version 6.12 SP1. A Kern 770 balance was used for weighing standards. In addition, a Millipore filter was used in the study.

The mobile phase consisted of ammonium acetate (0.5 M)–acetonitrile–methanol (50:15:35, v/v/v). The elution was isocratic for the first 6 min and was altered gradually to ammonium acetate (0.5 M)–acetonitrile–methanol (40:30:30, v/v/v) over 1 min. This composition was maintained for an additional 4 min. The initial eluent composition was restored in 5 min. The mobile phase was filtered through 0.45 μm membrane filter. The flow rate was set at 1.5 ml/min, the column temperature was 25 °C and the temperature of the autosampler was 15 °C. The injection volume was 20 μl.

2.3. Standard preparation

One hundred and sixty-seven milligrams of ranitidine HCl (equivalent to 150 mg ranitidine) was accurately weighed, dissolved in water and diluted to 5.0 ml (standard solution A). Fifty milligrams methylparaben and 12.5 mg propylparaben were dissolved in MeOH and diluted to 5.0 ml (standard solution B). One milliliter of standard solution A was mixed with 0.2 ml of standard solution B and the solution was diluted to 2.0 ml with the mobile phase.

2.4. Sample preparation

One hundred and fifty microliters of the oral liquid was passed through the SPE cartridge (Waters SPE HLB column) by gravity flow. The cartridge was conditioned with 2 ml of methanol and 2 ml of 10% methanol in water. One hundred and fifty microliters of the sample to be examined was applied on the column and the cartridge was washed with 150 μl of 10% acetonitrile in water. Ranitidine and parabens were eluted off the sorbent using 4.0 ml of a mixture of acetonitrile and water (60:40, v/v) and diluted to 5.0 ml with the mobile phase.

2.5. Validation studies

Accuracy, system precision/intermediate precision, linearity, selectivity and robustness of the method were checked. The samples for the linearity test were prepared by spiking placebo samples with weighed amounts of ranitidine, MP and PP. Solutions corresponding to each concentration level were prepared as described above in order to obtain the range of concentration as reported in Table 1.

Table 1
Validation data/linearity and accuracy/recovery study ($n = 5$)

Component	Concentration range (mg/ml)	Regression equation	Correlation coefficient	Recovery average (%)	Recovery R.S.D. (%)
Ranitidine	7.50–19.50	$y = 0.9898x + 0.1051$	0.9995	99.8	0.6
Methylparaben	0.50–1.30	$y = 0.9811x + 0.1930$	0.9997	99.6	0.8
Propylparaben	0.125–0.325	$y = 1.0032x + 0.0003$	0.9996	99.5	0.5

The precision of the method was assessed by determining the R.S.D. values of the analysis ($n = 6$) of the sample (100%) by three analysts, each one using a different chromatographic apparatus. The accuracy of the samples was checked by determining the concentration of the samples prepared for linearity.

The robustness of the method was proven by changing parameters of the analysis (column temperature and type of the analytical column). Also, the stability of the standard and sample solutions was examined.

3. Results and discussion

3.1. Method development

Different cartridges for the SPE were tested during method development in order to obtain satisfactory recovery of ranitidine, MP and PP. The SPE sorbents tested were [poly(divinylbenzene-co-*N*-vinylpyrrolidone)] (Oasis HLB, Waters), C18 and [poly(divinylbenzene-co-*N*-vinylpyrrolidone)] with cation-exchange sulfonic acid groups (Oasis MCX, Waters). The choice of the SPE sorbents was based on literary data. C18 [15] and Oasis HLB [14] cartridges have been reported to be suitable for the pretreatment of samples in the determination of parabens by HPLC and Oasis MCX (Waters) which are highly selective for basic drugs [22]. In the present work the SPE clean-up procedure (cartridge and eluent conditions) selected was the one reported by Pongcharoenkiat [14]. [Poly(divinylbenzene-co-*N*-vinylpyrrolidone)] SPE sorbent presented acceptable recoveries (>98%) of ranitidine, MP and PP in the same fraction.

The main problem in optimizing the chromatographic conditions was to achieve the coelution of ranitidine, MP and PP, the elution of PP within a reasonable time and simultaneously a good separation of ranitidine and MP. The British Pharmacopoeia 2004 [7] specifies a HPLC method for the determination of ranitidine in oral solution using a C18 column and a mixture of ammonium acetate 0.1 M: methanol, (15:85) and a UV detection at 320 nm. Using the above chromatographic system with UV detection at 254 nm, where the parabens can be detected, resulted in a poor resolution between ranitidine and MP and tailing peaks. Review of the literature justified the above findings, as it indicated that basic drugs such as ranitidine may show undesirable interactions (hydrogen binding and/or ion-exchange) with uncapped silanols of silica-based reversed phase materials [6]. These interactions give rise to tailing peaks, poor resolution and even complete retention of some solutes. Since buffers can mask silanol interaction, the chromatographic behavior of the analytes was improved by increasing the concentration of the ammonium acetate buffer in mobile phase. In the absence of ammonium acetate from the mobile phase, no resolution between ranitidine and MP was observed. The addition of ammonium acetate improved the behavior of ranitidine. The change of the concentration of ammonium acetate solution from 0.0 to 0.7 mol/l in mobile phase was investigated for the analytes retention and separation. The most satisfactory separation was achieved in the case of 0.5 M ammonium acetate (Fig. 1).

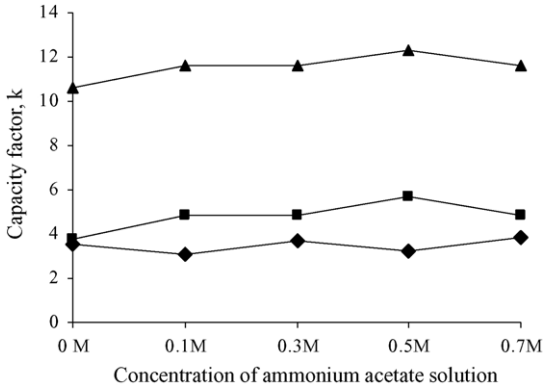

Fig. 1. The effect of the concentration of ammonium acetate solution on analytes retention: (♦) ranitidine; (■) MP; (▲) PP.

The influence of gradient elution on the analysis was also examined. In the case of isocratic elution, increased retention times were observed. Also, the peak of PP presented a high-tailing factor value (Fig. 2(b)). Based on the above findings a gradient elution system was selected.

A typical chromatogram of standard solution of ranitidine, MP and PP acquired by the developed HPLC method is presented in Fig. 2(a). The resolution factor between ranitidine–MP and MP–PP are 5.2 and 18.2, respectively.

3.2. Method validation

3.2.1. Linearity/range

Six solutions were prepared for the linearity test containing different concentrations of ranitidine, MP and PP in the range of 50–130% of the theoretical values (ranitidine 15 mg/ml, MP 1 mg/ml, PP 0.25 mg/ml). Each solution was injected five times and linear regression analysis of ranitidine, MP and PP nominal concentration versus measured concentration were calculated (Table 1).

3.2.2. System precision

The system precision was examined by analyzing six determinations of the same test concentration 100% (ranitidine 15 mg/ml, MP 1 mg/ml and PP 0.25 mg/ml). The relative standard deviation of the areas of each peak was found to be less than 0.7% (Table 2).

3.2.3. Intermediate precision

The precision of the method was assessed by determining the R.S.D. values of the analysis ($n = 6$) of the oral liquid containing 100% of the theoretical values of the active ingredient and the preservatives. Three analysts conducted the same test, each one using a different chromatographic system on a different day. The R.S.D. values were found to be less than 1.2% (Table 2), which demonstrates good precision of the method.

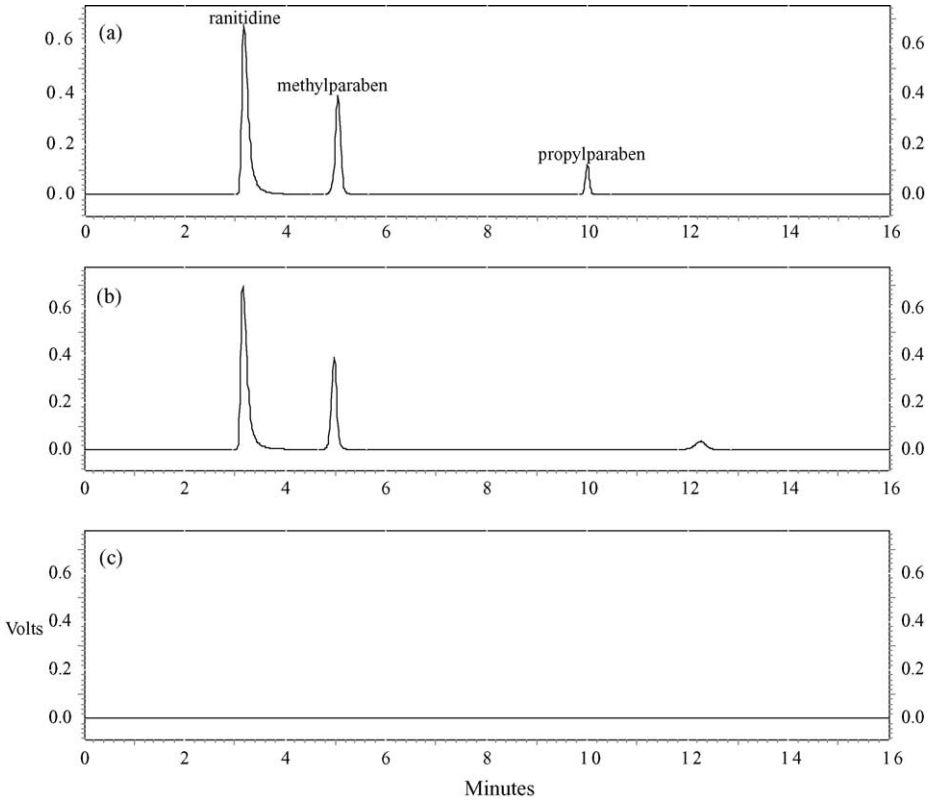

Fig. 2. Typical chromatogram obtained from injection of (a) extracted sample using gradient elution; (b) extracted sample using isocratic elution and; (c) extracted placebo sample using gradient elution.

3.2.4. Accuracy/recovery

The accuracy of the method was checked by evaluating the experimental concentration of the solutions prepared for the linearity test versus the nominal concentration. The percentage recovery of all three substances was greater than 99.0% as shown in Table 1.

3.2.5. Specificity and selectivity

The method was found to be selective since injection of the placebo solution confirmed the absence of interfering peaks at the retention times of the three examined substances at 254 nm (Fig. 2(c)). In addition, the spectrum of each substance (190–370 nm) in the sample solution is identical to the spectrum received by the standard solution. These results demonstrate that there was no interference from other materials in the liquid pharmaceutical formulation and therefore confirm the specificity of the method.

3.2.6. Stability of analytical solutions

Sample and standard solutions were analyzed immediately after preparation and after storage at room temperature and darkness for 24 h. The response of the three substances was not significantly altered over this period as shown in Table 2.

3.2.7. Robustness

Several parameters of the analysis were purposely altered in order to determine the robustness of the method. The temperature of the column was set at 23 °C and 30 °C and the

Table 2
Method validation results

Validation step	Parameters	Ranitidine (%)	Methylparaben (%)	Propylparaben (%)	Acceptance criteria (*X*)
System precision	R.S.D. (%)[a]	0.64	0.15	0.13	<2
Intermediate precision					
Analyst 1	R.S.D. (%)[a]	0.62	0.18	0.17	<2
Analyst 2	R.S.D. (%)[a]	0.30	0.38	0.57	<2
Analyst 3	R.S.D. (%)[a]	0.45	0.60	0.62	<2
Analysts 1–3	R.S.D. (%)	0.67	1.14	0.28	<2
Standard stability (24 h)	Change in response factor[b]	0.18	0.10	1.03	<2
Sample stability (24 h)	Change in response factor[b]	0.55	0.49	1.39	<2

[a] $n = 6$.
[b] $n = 3$.

sample preparation 100% was analyzed five times. The estimated concentration and the retention times of the analytes did not change significantly.

Also, the analysis was carried out on different brands of reversed stationary phases. The system precision was checked using Lichrospher 100 RP-18 and ODS Hypersil stationary phases. The R.S.D. values using the above stationary phases were less than 1.0%. Furthermore, satisfactory separation and symmetric peaks were achieved using any of the above stationary phases.

4. Conclusions

The proposed HPLC method employing SPE for sample preparation is simple and reliable for the simultaneous determination of ranitidine, MP and PP in oral pharmaceutical solutions. The method also provides an efficient clean-up procedure of the pharmaceutical formulation. The method was validated and the results obtained were within acceptable limits. The determination of MP, PP and ranitidine by SPE followed by HPLC analysis yielded well-resolved peaks, excellent recovery (>99.0%) and good precision (R.S.D. <2.0%). This method can be successfully applied for the identification, quantitative analysis and stability test of ranitidine, MP and PP in liquid pharmaceutical formulations.

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