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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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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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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