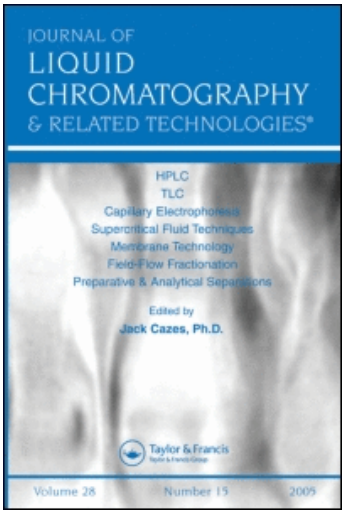

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G. R. Padmanabhan[a]; J. Smith[a]; N. Mellish[a]; G. Fogel[a]
[a] Department Suffern, Ciba-Geigy Corporation, Pharmaceuticals Division Research & Development, New York

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HIGH PRESSURE LIQUID CHROMATOGRAPHIC DETERMINATION OF PARABENS IN PHARMACEUTICAL PREPARATIONS CONTAINING HYDROXYQUINOLINES

G. R. Padmanabhan*, J. Smith, N. Mellish, and G. Fogel
Ciba-Geigy Corporation, Pharmaceuticals Division
Research & Development Department
Suffern, New York 10901

ABSTRACT

A high pressure liquid chromatographic (HPLC) procedure for the analysis of methyl paraben (MP) and propyl paraben (PP) in pharmaceutical preparations containing a halogenated hydroxyquinoline (HHQ) is described. The method involves a separation of the phenolic constituents, MP, PP and HHQ with a Bio-Rad AG® 1-X8 anion exchange resin, elution of the phenols with methanol after acidification and a reverse phase HPLC separation of the parabens using methanol - pH 6.5 buffer (60/40) mobile phase, a 30 cm x 3.9 mm (i.d.) column packed with Waters μBondapak C_{18} packing and a guard column packed with Waters Bondapak C_{18}/Corasil packing. Recovery, precision, specificity and interference data along with the application of the proposed method for some commercial formulations both with and without a hydroxyquinoline are described.

INTRODUCTION

Methyl and propyl parabens are used extensively as preservatives in pharmaceutical, food and cosmetic preparations in order to prevent the growth of microbials (1). Several analytical methods have been reported in the literature for the analysis of the parabens. These methods include colorimetry (2-3), gas chromatography (4-5) ion-exchange (6-7) and reverse phase HPLC (8-11), partition chromatography (12), adsorption chromatography

(13-14), ultraviolet spectrophotometry (15), and thin-layer chromatography (16-17). However, when we employed the above methods for the analysis of a pharmaceutical lotion preparation which also contained the active ingredients iodochlorhydroxyquin and hydrocortisone in addition to the parabens, none of the above methods could be employed successfully due to one or more of the following reasons: 1. high iodochlorhydroxyquin/parabens concentration ratio 2. phenolic properties of both parabens and iodochlorhydroxyquin 3. high polarity of iodochlorhydroxyquin (18) 4. weak basicity of iodochlorhydroxyquin 5. poor stability of parabens in basic solutions 6. formation of emulsion during clean-up procedure due to the excipients present in the sample 7. relative high solubilities of parabens in water and 8. presence of interfering components in some of the excipients such as lanolin used in the lotion formulation (19). In this publication we are reporting a procedure which is based on a preliminary ion-exchange separation of the parabens and iodochlorhydroxyquin from the formulation, elution of these compounds from the column, precipitation and removal of a majority of iodochlorhydroxyquin at a pH of 6.5 and a subsequent HPLC separation and quantitation of the individual parabens.

MATERIALS AND METHODS

Apparatus

A modular HPLC instrument with a Waters Model 6000A solvent delivery system, a Waters Model 440 absorbance detector (fixed wavelength at 254 nm) and a Valco injection valve with 100 µL loop was used. For HPLC separation, a Waters µBondapak C_{18} column, 30 cm x 3.9 mm (i.d.) and a guard column packed with Waters Bondapak C_{18}/Corasil were used.

Mobile Phase

Mix thoroughly 600 mL of methanol and 400 mL of a pH 6.5 buffer solution. The buffer solution was prepared by adding the

appropriate amount of 0.1N sodium hydroxide to 250 mL of 0.1N monobasic sodium phosphate to obtain a pH value of 6.5 and then adjusting the volume to 1000 mL with water.

Chromatographic Conditions

A mobile phase flow rate of 1 mL/minute (isocratic) was used for the study. The column was maintained at room temperature and the detector sensitivity was maintained at 0.1 AUFS for MP and at 0.04 for PP.

Standard Preparation

Prepare a standard methanolic stock solution containing 2.25 μg of MP/mL and 1.25 μg of PP/mL. Dilute 25 mL of the stock solution to 50 mL with the pH 6.5 buffer solution. Filter and inject 100 μL into the column.

Procedure

Take 1.5 g of AG 1-X8 (chloride form) analytical grade anion exchange resin (Bio-Rad Laboratories) in a 25 cm x 10.5 mm (i.d.) glass chromatographic column equipped with a 200 mL reservoir at the top and a stopcock at the bottom and convert the resin into the hydroxide form with 1N sodium hydroxide. Wash the column with water and methanol. Extract an amount of lotion or cream sample equivalent to about 0.7 mg of total parabens three times with 15 mL of methanol, filter, if necessary, collect the clear liquid and dilute to 50 mL with methanol. Disperse the ointment sample with 25 mL of ether, add 15 mL of 0.1 N HCl, shake and collect the ether layer. Repeat the extraction twice with 25 mL of ether. Combine the ether layers and evaporate the ether. Dissolve the residue in 50 mL of methanol. Pass a 25 mL aliquot of the sample solution through the ion-exchange column and wash the column successively with 100 mL of methanol, 10 mL of 0.5N hydrochloric acid and 10 mL of water. Discard the eluants. Pass 100 mL of methanol through the column, collect the eluant and adjust the final volume to 100 mL. Dilute 25 mL of the sample solution to 50 mL with pH 6.5 buffer, centrifuge an aliquot and filter.

Inject 100 µL into the HPLC column. Compare the peak heights (or the area) of the sample and the standard solutions and calculate the amount of MP and PP in the sample.

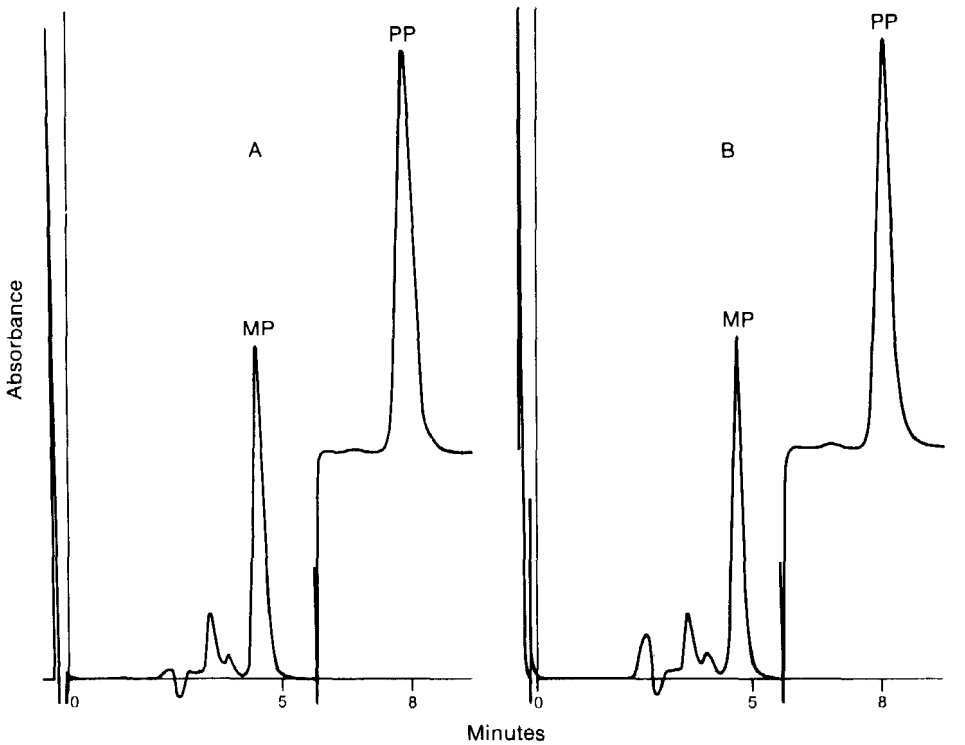

FIGURE 1

A. A typical chromatogram showing the separation of MP and PP from the analysis of a 1 g sample of a iodochlorhydroxyquin-hydrocortisone lotion formulation using the method described in the text. The detector sensitivity was changed from 0.1 AUFS to 0.04 AUFS after the elution of MP.

B. Same as A except for the addition of 0.087 mg of parahydroxy benzoic acid to the sample before the analysis by the proposed method.

RESULTS AND DISCUSSIONS

A chromatogram obtained from a typical analysis of a lotion sample labelled to contain 0.07% of total parabens, 1% hydrocortisone and 3% iodochlorhydroxyquin is shown in Figure 1. Interference values of 2% and < 1% respectively were obtained for MP and PP when a paraben placebo lotion sample which contained hydrocortisone and iodochlorhydroxyquin was analyzed by the proposed method. Samples of the placebo lotion samples were spiked with MP and PP at a level of approximately 80%, 100% and 120% of their amount present in a typical iodochlorhydroxyquin-hydrocortisone lotion sample and then analyzed by the proposed method. The results, shown in Table 1, indicate that the recovery of MP is within 101-104% range and the recovery of PP within 97-99% range.

TABLE 1

Recovery and Linearity Data For
Iodochlorhydroxyquin-Hydrocortisone Lotion Formulation

Amount of Placebo g	Amount Added mg		Amount Found mg		Recovery %	
	MP	PP	MP	PP	MP	PP
1.004	0.331[1]	0.214[1]	0.346	0.212	104	99
0.952	0.414[2]	0.268[2]	0.419	0.260	101	97
1.014	0.497[3]	0.322[3]	0.515	0.318	104	99

MP = Methyl Paraben PP = Propyl Paraben

[1]Approx. 80% of Label

[2]Approx. 100% of Label

[3]Approx. 120% of Label

TABLE 2

Parabens in a Iodochlorhydroxyquin-Hydrocortisone Lotion[5]

Sample	Current Method[4] % Total Parabens	Proposed HPLC Method		
		% MP	% PP	% Total Parabens
A[1]	0.07	0.044	0.024	0.068
A[2]	--	0.044	0.024	0.068
B[1]	0.066	0.044	0.022	0.066
B[2]	--	0.042	0.022	0.064
C[1]	0.070	0.046	0.025	0.071
D[3]	0.020[6]	0.044	0.023	0.067

[1] = Chemist #1 Lab 1
[2] = Chemist #2 Lab 1
[3] = Chemist #3 Lab 2
[4] = Recovery of Spiked Placebo was < 100% by this Alumina Column - UV method. However, the standard is treated in the same way as the sample in this method.
[5] = Label claim: total parabens = 0.07%.
[6] = Probably due to the poor reproducibility of the alumina employed

The results obtained from the analysis of four different batches of a commercial iodochlorhydroxyquin-hydrocortisone lotion are shown in Table 2. The results indicate that the proposed method is more accurate than a alumina adsorption column - UV combination method employed currently for this product. The results also show that the inter- and intra-laboratory reproducibilities of the proposed method are satisfactory.

In addition to the potential binding of parabens with the excipients present in some of the formulations (20-22), parabens are also known to hydrolyze to parahydroxybenzoic acid (PHBA) particularly in the basic solutions (23). In order to establish the specificity of the proposed method for MP and PP in presence of PHBA, a lotion sample containing MP, PP, iodochlorhydroxyquin

TABLE 3

Parabens in Some Typical Formulations

#	Sample	Claim, %	Found, %	
		Total Parabens	MP	PP
1	Crotamiton Cream	0.4	0.25	0.14
2	Flumethasone Pivalate Cream	0.04	0.021	0.014
3	HC Cream	NA*	0.19	0.09
4	HC Cream + 2% CQ added[1]	NA*	0.18	0.10
5	HC Lotion	NA	0.14	0.02
6	HC - ICHQ Cream	NA*	0.12	0.05
7	HC - ICHQ Ointment	NA	ND	ND
8	HC - DIHQ Cream	0	ND	ND
9	HC - DIHQ Cream	0.072[2]	0.044	0.027

[1]Sample #4 is same as sample #3 with 2% CQ added.
[2]Sample #9 is same as sample #8 with 0.044% MP and 0.028% PP added.

HC - Hydrocortisone; CQ - Chlorquinaldol
ICHQ - Iodochlorhydroxyquin; DIHQ - Diiodohydroxyquin

NA* - Parabens present in formulations; amount not indicated.
NA - Information not available.
ND - No parabens detected by the proposed method.

and hydrocortisone was analyzed with and without added PHBA. The results shown in Figure 1 indicate that PHBA does not interfere with the analysis of MP and PP. It has to be pointed out, however, that the proposed method as such cannot be employed for the determination of PHBA due to the fact PHBA is retained on the HPLC column under the conditions employed. If the quantitation of PHBA is desired, one can accomplish this by substituting water for pH 6.5 buffer in the mobile phase of HPLC and in the final step of the sample preparation. This modification will result in the elution of PHBA. However the reproducibility of MP and PP quantitations are not satisfactory with the mobile phase system that is not buffered.

In addition to the analysis of iodochlorhydroxyquin lotion, the proposed method was also applied to the analysis of parabens in some other semi-solid formulations containing one or more of the following: iodochlorhydroxyquin, diiodohydroxyquin, chlorquinaldol, crotamiton, flumethasone pivalate and hydrocortisone. The results, included in Table 3, indicate that the proposed method is applicable to these formulations as well.

The proposed method has two minor disadvantages, first the clean-up step is somewhat time consuming. However, the analysis time can be shortened by carrying out the complete analysis in one step using a short ion-exchange column in front of the HPLC column and employing a series of valves in order to automate the clean-up steps (24-26). The other minor disadvantage of the method is the need for a periodic replacement of the guard column included in the HPLC step. The guard column was included in order to eliminate the potential drift in the baseline due to the elution of iodochlorhydroxyquin particularly after repeated injection of the sample solution.

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