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High performance liquid chromatographic determination of phenoxetol, methyl paraben, ethyl paraben, *n*-propyl paraben, *iso*-butyl paraben, *n*-butyl paraben and croconazole·HCl

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Abstract

A high performance liquid chromatographic technique has been used to determine phenoxetol, methyl paraben, ethyl paraben, *n*-propyl paraben, *iso*-butyl paraben, *n*-butyl paraben and croconazole·HCl. The method developed involves isocratic, reversed phase chromatography. The range of quantitation was found to be 90–135.0 μg ml^{-1} for phenoxetol, 2.0–30.0 μg ml^{-1} for methyl paraben, 0.5–7.5 μg ml^{-1} for ethyl paraben, 0.3–4.5 μg ml^{-1} for *n*-propyl paraben, 0.3–4.5 μg ml^{-1} for iso-butyl paraben, 0.5–7.5 μg ml^{-1} for *n*-butyl paraben and 20.0–300 μg ml^{-1} for croconazole·HCl. Linear regression analysis of the data demonstrates the adequate performance of the method in terms of precision and accuracy.

Keywords: *n*-Butyl paraben; *iso*-Butyl paraben; Croconazole·HCl; Ethyl paraben; Isocratic elution; Methyl paraben; *n*-Propyl paraben; Phenoxetol; Reversed-phase liquid chromatography

1. Introduction

Croconazole hydrochloride 1-[1-[*o*-(*m*-chlorobenzyloxy)-phenyl]vinyl]-1*H*-imidazole hydrochloride, a new imidazole-type antifungal agent synthesized at Shionogi Research Laboratories [1], has a broad antifungal spectrum and strong antifungal activity [2]. It exerts antifungal activity by damaging the cell membrane permeability of fungus and inhibiting the synthesis of ergosterol, like other imidazole-type antifungal agents [3].

Croconazole and its metabolites have been identified in biological samples using thin layer chromatography (TLC), gas–liquid chromatography (GLC), mass spectrometry (MS) [4,5], high performance liquid chromatography, (HPLC) and NMR [6].

Croconazole·HCl has been formulated in cream and gel forms [1] containing a suitable proportion of preservatives, such as esters of *p*-hydroxybenzoic acid. Preservatives are added to dosage forms to protect them from microbial contamination in the formulation. Efficacy of the preservatives added in the dosage form is tested by the preservative efficacy test [7].

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An HPLC method using μ-Bonda Pak C_{18} and an eluent based on 0.05 M KH_2PO_4 and acetonitrile has been developed to simultaneously determine the active ingredient, i.e. croconazole·HCl, and the inactive ingredients i.e. homologus series of hydroxy benzoic acids, in a pharmaceutical preparation with very simple sample treatment prior to injection. Details of the method and its validation are reported here.

2. Experimental

2.1. Materials

Potassium dihydrogen phosphate,—guaranteed reagent tested according to International Standardization Organization, Merck specification—methanol, acetonitrile and lichro Solv were obtained from Merck. Standards were obtained, courtesy of NIPA Laboratories UK. All stock solutions were prepared by dissolving reference standards of phenoxetol, methyl paraben, ethyl paraben, *n*-propyl paraben, *iso*-butyl paraben, *n*-butyl paraben and croconazole·HCl in methanol.

2.2. HPLC method

The chromatograph used in this study consisted of a LC-10AS pump (Shimadzu, Japan), an SIL-9A injector, an SPD-10A detector and a C-R4A integrator. μ-Bonda Pak C_{18} 300 mm × 3.9 mm i.d. columns were used, protected by an ODS (octadecyl silane) pre-column.

A degassed and filtered mixture of 0.05 M potassium dihydrogen phosphate, pH 3.50, and acetonitrile (65:35 v/v) was used as eluent. The flow rate was maintained at 1.0 ml min^{-1}. Detection was performed at 254 nm. The run time for each analysis was 30 min. All separations were carried out at ambient temperature.

2.3. Preparation of standard solutions

20 mg of methyl paraben and 90 mg of phenoxetol, accurately weighed, were transferred to a 100 ml volumetric flask. The substances were dissolved in and made up to volume with methanol. This was designated as solution "A".

25 mg of ethyl paraben, 15 mg of *n*-propyl paraben, 15 mg of *iso*-butyl paraben and 25 mg of *n*-butyl paraben were accurately weighed and transferred to a 100 ml volumetric flask. The standards were dissolved in and made up to volume with methanol. This was designated as solution "B".

100 mg of croconazole·HCl, (Shionogi reference standard, Shionogi & Co., Ltd., Osaka, Japan) accurately weighed was transferred to a 50 ml volumetric flask. It was dissolved in and made up to volume with methanol. This was designated as solution "C".

10 ml of solution A, 2 ml of solution B and 10 ml of solution C were transferred to a 100 ml volumetric flask. The contents of the flask were diluted to volume with methanol. This was the reference standard solution. The solution was filtered through a 0.45 μm membrane filter before injection into the liquid chromatograph.

2.4. Preparation of test solution

A test solution containing methyl paraben (20 μg ml^{-1}), phenoxetol (90 μg ml^{-1}), ethyl paraben (5.0 μg ml^{-1}), *n*-propyl paraben (3.0 μg ml^{-1}), *iso*-butyl paraben (3.0 μg ml^{-1}), *n*-butyl paraben (5.0 μg ml^{-1}) and croconazole·HCl (200 μg ml^{-1}), was prepared using methanol as diluent. The solution was filtered through a 0.45 μm membrane filter before injection into the liquid chromatograph.

2.5. Observed retention times

The observed retention times of the reference compounds were found to be 6.2, 7.1, 10.0, 16.0, 20.0, 26.0 and 27.5 min for phenoxetol, methyl paraben, ethyl paraben, *n*-propyl paraben, croconazole·HCl, *iso*-butyl paraben and *n*-butyl paraben respectively. The variation in retention time was found to be $\pm$ 5% of the observed retention time.

2.6. Calculations

In the routine analysis of a pharmaceutical preparation, an external standard method, i.e. the

bracketing of test solution with the reference standard solution, was carried out to quantify croconazole·HCl and preservatives.

3. Results and discussions

Individual test standards containing 10, 20, 50, 80, 90, 100, 120 and 150% of the theoretical assay concentration of each of the compounds were examined by the HPLC method and the responses measured. In each case, i.e. test and standard, a linear relationship between peak height and concentration was observed. Fig. 1 shows a sample chromatogram obtained from the analysis of an actual formulation. The recovery data (Table 2) clearly show that other consituents of the formulation are not interfering with the assay.

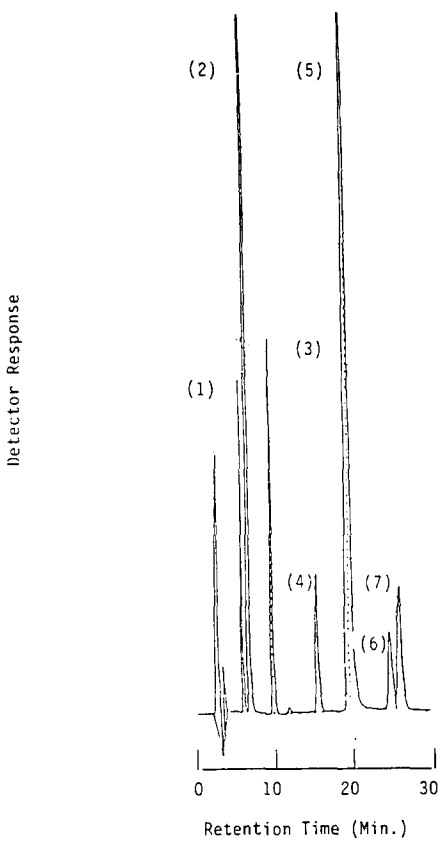

Fig. 1. Chromatogram of test solution containing 90 μg ml^{-1} of phenoxetol (1), 20 μg ml^{-1} of methyl paraben (2), 5.0 μg ml^{-1} of ethyl paraben (3), 3.0 μg ml^{-1} of *n*-propyl paraben (4), 200 μg ml^{-1} of croconazole·HCl (5), 3.0 μg ml^{-1} of *iso*-butyl paraben (6) and 5.0 μg ml^{-1} of *n*-butyl paraben (7).

Table 1
Regression analysis of the calibration data[a]

Compound	Slope ($\pm$SD)	Intercept ($\pm$SD)	r^2
Phenoxetol	895.55 $\pm$ 9.70	113.28 $\pm$ 894.71	0.999
Methyl paraben	6184.32 $\pm$ 119.16	−2485.44 $\pm$ 10986.0	0.999
Ethyl paraben	1401.47 $\pm$ 16.37	−457.03 $\pm$ 1509.72	0.999
n-Propyl paraben	780.09 $\pm$ 11.71	−372.37 $\pm$ 1079.71	0.999
Iso-butyl paraben	6781.09 $\pm$ 162.18	−839.25 $\pm$ 14952.3	0.999
n-Butyl paraben	748.71 $\pm$ 23.60	−1849.75 $\pm$ 2176.3	0.999
Crocanazole·HCl	1208.11 $\pm$ 16.14	−1306.99 $\pm$ 1487.98	0.999

[a] Averages of four experiments.

Regression analysis of data ($n = 4$) for each component gave the values for solpe, intercept and correlation coefficient for each calibration curve (summarized in Table 1).

The validity of listed regression data was tested by the assay of an authentic mixture containing known quantities of phenoxetol, methyl paraben, ethyl paraben, *n*-propyl paraben, *iso*-butyl paraben, *n*-butyl paraben and croconazole·HCl. The result showed good accuracy (as revealed by the percentage recovery; see Table 2). The recovery data were generated by the assay of solutions containing 80, 100 and 120% of the theoretical assay concentration. Methanol was used as diluent because the sample, formulated as a cream, gives good recovery when tested against the standards dissolved in methanol.

The external standard method is routinely used as a quality control procedure for the analysis of the formulation containing croconazole and preservatives. So far more than 100 samples have been analysed on the column without any significant deterioration in column performance (Table 3). The minimum column efficiency required to carry out this method was found to be 6000 plates.

Table 2
Simultaneous determination of phenoxetol, methyl paraben, ethyl paraben, *n*-propyl paraben, *iso*-butyl paraben, *n*-butyl paraben and croconazole·HCl by the proposed HPLC method

Compound	Amount added (μg ml^{-1})	Amount found (μg ml^{-1})	Recovery (%)
Phenoxetol	69.88	70.706	101.18
	87.27	87.18	99.89
	104.52	103.97	99.47
Mean (± RSD)			100.14 (± 0.90)
Methyl paraben	16.04	16.2	101.02
	20.35	20.445	100.46
	24.53	24.44	99.63
Mean (± RSD)			100.37 (± 0.70)
Ethyl paraben	4.04	4.07	100.82
	5.02	5.04	100.41
	5.98	5.97	99.88
Mean (± RSD)			100.46 (± 0.83)
n-Propyl paraben	2.43	2.44	100.69
	3.03	3	99.24
	3.57	3.58	100.19
Mean (± RSD)			100.06 (± 0.69)
iso-Butyl paraben	2.44	2.45	100.2
	3.03	3	99.24
	3.57	3.58	100.19
Mean (± RSD)			99.88 (± 0.38)
n-Butyl paraben	4.02	4.03	100.39
	5.04	5.02	99.68
	6.06	6.04	99.66
Mean (± RSD)			99.91 (± 0.41)
Crocanazole·HCl	161.76	164.56	101.73
	202.3	203.51	100.59
	241.8	242.5	100.28
Mean (± RSD)			100.86 (± 0.75)

The separation of all seven components in a single run justifies the use of HPLC and the validation data convincingly demonstrate the adequate performance of the proposed method, which is required for the assay of pharmaceutical prepartions.

Table 3
Variation of column performance with time

Column	Run No.	Number of theoretical plates[a]
μ-Bonda Pak C_{18}	1	8856
μ-Bonda Pak C_{18}	100	7082

[a] Calculated with respect to the *n*-propyl paraben peak.

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