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Note

Quantitative gas and thin-layer chromatographic determination of methylparaben in pharmaceutical dosage forms

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Methyl *p*-hydroxybenzoate (methylparaben) is one of the most important pharmaceutical preservatives. The determination of methylparaben in multi-component pharmaceutical preparations is complicated by the fact that its total concentration seldom exceed 0.1% of the formulation. The possibility that it can interact chemically with active ingredients[1] led to a need for a rapid and reliable quantitative method for the determination of methylparaben in dosage forms.

Some methods for the quantitative and semiquantitative determination of parabens have been published[2–9], but less attention has been paid to the pre-treatment of dosage forms. Quantitation of preservatives in pharmaceutical preparations poses a considerable analytical problem. Owing to the complex matrix present and the high concentrations of excipients, even chromatographic quantitation cannot be carried out without an extensive preliminary cleanup. Previously reported multiple extraction methods are time consuming and often give poor recoveries.

In the work described here a single extraction step precedes the quantitation. Extrelut (Merck) columns were used. The aqueous mixtures of the preparations were added to the column and the methylparaben was eluted with diethyl ether or ethanol, depending on the composition of the formulation. The interfering excipients can be eliminated by this simple procedure and at the same time the recovery of methylparaben is very good. The paraben content of the eluate can be determined quantitatively by either gas (GC) or thin-layer chromatography (TLC).

To demonstrate the feasibility of the methods for the determination of methylparaben in dosage forms, the procedures were applied to the analysis of some mucilages, syrups and ointments listed in the Hungarian Pharmacopoeia.

EXPERIMENTAL

All reagents used were of analytical-reagent grade and the drug substances were of pharmacopoeial standard.

Compositions of the formulations examined

Mucilages. Methylcellulose Mucilage: methylcellulose 25 g, glycerine 100 g, methyl *p*-hydroxybenzoate 1 g, ethanol 9 g, water 865 g.

Hydroxyethylcellulose Mucilage: hydroxyethylcellulose 30 g, methyl *p*-hydroxybenzoate 1 g, ethanol 9 g, water 960 g.

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Methylcellulose Hydrogel: methylcellulose 50 g, glycerine 100 g, methyl *p*-hydroxybenzoate 1 g, ethanol 9 g, water 840 g.

Syrups. Laxative Syrup: senna leaf 50 g, foeniculum fruit 5 g, methyl *p*-hydroxybenzoate 1 g, ethanol 9 g, dried sodium sulphate 50 g, sucrose 500 g, water to 1000 g.

Liquorice Syrup: liquorice fluid extract* 200 g, simple syrup 790 g, methyl *p*-hydroxybenzoate 1 g, ethanol 9 g.

Ointments. Anionic Hydrophilic Ointment: anionic emulsifying ointment** 400 g, methyl *p*-hydroxybenzoate 1 g, ethanol 9 g, water 590 g.

Stearin Ointment: cetostearyl alcohol 45 g, stearin 100 g, sodium laurylsulphate 5 g, methyl *p*-hydroxybenzoate 1 g, ethanol 9 g, sorbitol 35 g, glycerine 100 g, water to 1000 g.

Pre-treatment of the samples

To 5 g of mucilage or 2.5 g of syrup or ointment were added 10 ml of 0.01 *M* hydrochloric acid and 20 ml of water. The mixture was poured on to an Extrelut column, which was aspirated with a water-jet pump until the liquid reached the bottom of the column, and after 20 min was eluted with three 40-ml portions of diethyl ether (for Anionic Hydrophilic Ointment with two 40-ml portions of 96% ethanol).

Gas chromatographic assay

To the combined, filtered (through cotton–anhydrous sodium sulphate) ethereal eluate an ethereal, and to the combined alcoholic eluate an alcoholic, internal standard solution containing about the same amount of 2-naphthol as methylparaben in the eluate was added. The solution was evaporated to dryness on a water-bath. The residue was dried over silica gel and dissolved in 0.5 ml of N,O-bis(trimethylsilyl)acetamide (BSA) and a 0.2-μl volume was injected into the GC column.

A Carlo Erba Model GV gas chromatograph equipped with a flame-ionization detector was used. The column was a glass tube (2 m × 3.0 mm I.D.) packed with 3% OV-225 on silanized Chromosorb W (100–120 mesh). Nitrogen was used as the carrier gas at a flow-rate of 50 ml/min and the column was operated at 160°C. Chromatograms were recorded on a Speedomax Model G recorder and integrated by a Carlo Erba Model 75 electronic integrator. The chart speed was 12.7 mm/min.

Thin-layer chromatographic assay

The combined and filtered (through cotton–anhydrous sodium sulphate) ethereal eluate or the combined alcoholic eluate was evaporated to dryness on a water-bath. The residue was dissolved in 5.00 ml of methanol. A 5-μl (mucilages) or a 10-μl volume (syrups or ointments) of each of three solutions, the solution of the eluate and two methanolic standard solutions containing the expected amount of methyl *p*-hydroxybenzoate were separately applied on a DC Alufolien Kieselgel 60 F-254

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* Liquorice fluid extract: crude liquorice dry extract 500 g, 10% ammonia solution 10 g, water 460 g, methyl *p*-hydroxybenzoate 3 g, ethanol 27 g.

** Anionic emulsifying ointment: sodium laurylsulphate 40 g, water 15 g, cetostearyl alcohol 360 g, liquid paraffin 200 g, vaselinum album 400 g.

(Merck) plate. The developing solvent was prepared by thoroughly shaking 70 ml of *n*-pentane, 30 ml of diethyl ether and 100 ml of water and, after separation of the layers, shaking the organic layer with 5 ml of 85% formic acid, and the upper layer was used. The plates were developed to a height of 150 mm in a pre-saturated chamber. The chromatograms were air dried at room temperature and examined at 254 nm. Methylparaben quenched the fluorescent background of the plate and the R_F value was 0.35. The spots were scanned with a Camag TLC scanner in the reflectance mode using a 258-nm filter. Densitograms were recorded on an SP 4100 computing integrator and the peak heights were measured.

RESULTS AND DISCUSSION

Satisfactory separations and good recoveries were achieved by using the Ex-

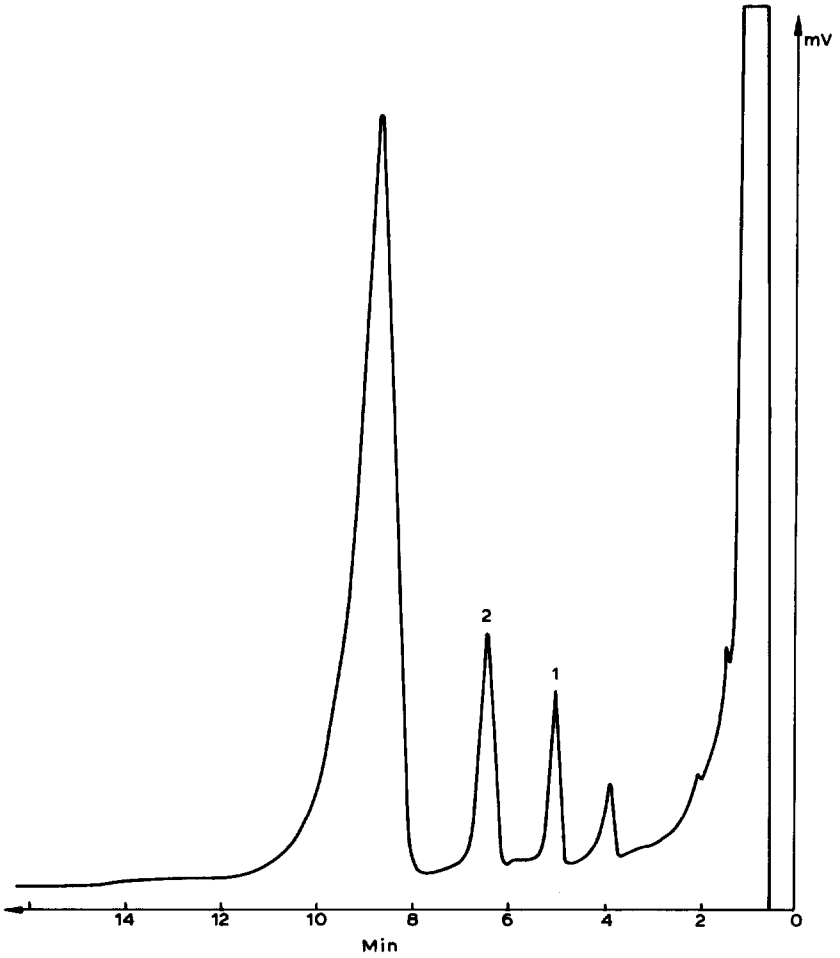

Fig. 1. Chromatogram of Stearin Ointment. Peaks: 1 = silylated methyl *p*-hydroxybenzoate; 2 = silylated 2-naphthol. Column: 2.0 m × 3 mm I.D., 3% OV-225 on silanized Chromosorb W. Temperatures: injector, 180°C; oven, 160°C; detector, 180°C. Nitrogen flow-rate, 50 ml/min.

trelut column. Excipients and components of the dosage forms that dissolve in diethyl ether or ethanol do not interfere with the subsequent GLC or TLC measurement.

The hydroxy group present in the paraben molecule causes irreversible adsorption on the GLC column, resulting in non-linear and variable quantitative results. This problem can be remedied by converting methyl *p*-hydroxybenzoate into a less polar derivative before it enters the column. Wilcox[8] reported the suitability of some higher diazoalkanes with boron trifluoride catalysis for the preparation of the alkyl ether derivatives. Donato[6] and Lach and Sawardeker[7] converted the parabens into their trimethylsilyl (TMS) ethers. The official assay of parabens in the USP XX[10] is also based on gas chromatography of the TMS ethers.

Higher diazoalkanes are not commercially available and their preparations are time consuming and tedious.

BSA has been found to be the most suitable reagent for derivatization of methylparaben as the TMS derivative is produced without any detectable by-products and the process is rapid with good reproducibility. Methyl *p*-hydroxybenzoate is readily soluble in pure BSA. The use of pyridine as solvent and the treatment of the paraben with a mixture of silylation agents as described in USP XX[10] was found to be superfluous.

Of a number of stationary phases tested, OV-225 gave the most symmetrical peaks. 2-Naphthol was chosen as the internal standard because its retention time is close to that of methylparaben and it is absent from pharmaceutical systems.

Fig. 1 shows a gas chromatogram obtained from one of the investigated dosage forms.

For the quantitative evaluation of gas chromatograms the internal standard method was used. The ratio of the area of the methylparaben peak to that of the internal standard peak was measured. Detector response factors relative to the internal standard were determined previously. Detector response linearities for methyl *p*-hydroxybenzoate and 2-naphthol were confirmed in the concentration range 0.5–15 mg/ml.

Accuracy and precision data for the GC method are given in Table I.

TABLE I

ACCURACY AND PRECISION OF THE GAS CHROMATOGRAPHIC METHOD

Experiments were carried out on laboratory-prepared preparations. The means, relative standard deviations and 90% confidence limits were determined by the analysis of two samples, each of which was chromatographed six times.

Dosage form	*Weight of methylparaben in the sample (mg)*	*Mean methylparaben found in the sample (mg)*	*Mean recovery (%)*	*S, (%)*	*90% confidence limits (mg)*
Methylcellulose Mucilage	5.90	5.89	99.83	0.83	5.89 ± 0.03
Hydroxyethylcellulose Mucilage	5.09	4.99	98.04	1.53	4.99 ± 0.04
Methylcellulose Hydrogel	5.00	4.75	95.00	0.49	4.75 ± 0.01
Laxative Syrup	2.50	2.51	100.40	1.81	2.51 ± 0.02
Liquorice Syrup	3.67	3.60	98.20	1.18	3.60 ± 0.02
Anionic Hydrophilic Ointment	2.49	2.49	100.08	0.74	2.49 ± 0.01
Stearin Ointment	2.53	2.49	98.58	1.02	2.49 ± 0.01

TABLE II

ACCURACY AND PRECISION OF THE THIN-LAYER CHROMATOGRAPHIC METHOD

Experiments were carried out on laboratory-prepared preparations. The means, relative standard deviations and 90% confidence limits were determined by the analysis of two samples, each of which was chromatographed six times

Dosage form	*Weight of methylparaben in the sample (mg)*	*Mean methylparaben found in the sample (mg)*	*Mean recovery (%)*	S_r *(%)*	*90% confidence limits (mg)*
Methylcellulose Mucilage	5.00	4.82	96.32	0.38	4.82 ± 0.31
Hydroxyethylcellulose Mucilage	5.00	4.82	96.38	0.26	4.82 ± 0.21
Methylcellulose Hydrogel	5.01	4.82	96.17	0.45	4.82 ± 0.37
Laxative Syrup	2.50	2.42	96.65	0.44	2.42 ± 0.36
Liquorice Syrup	2.50	2.38	95.20	0.16	2.38 ± 0.13
Anionic Hydrophilic Ointment	2.51	2.37	94.57	0.71	2.37 ± 0.58
Stearin Ointment	2.50	2.37	94.81	0.60	2.37 ± 0.50

As an alternative technique, a rapid TLC method suitable for routine use was developed for the identification and quantitation of methyl *p*-hydroxybenzoate in dosage forms. The spot of methylparaben separated from excipients and active ingredients was measured by densitometry in the reflectance mode at 258 nm.

Peak heights were evaluated and a linear relationship between concentration and peak height was established for concentrations of methylparaben between 1 and 10 μg. The results were calculated by comparing the peak height of methylparaben with those of the standard solutions. The peak heights of the standard solutions were taken as 100%.

Table II shows the accuracy and precision data of the TLC method.

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