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THE PRESERVATION OF TABLETS AGAINST MICROBIAL SPOILAGE

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There has been an increasing interest in the microbial content of non-sterile pharmaceutical products during the past decade, such that standards now appear for these as well as for recognized sterile preparations (Diagram 1). Although oral preparations might not rank high as microbial hazards the events of the past years have indicated that tablets can contain surprisingly high levels of microorganisms including certain pathogens. Kallings et al[1] traced an outbreak of

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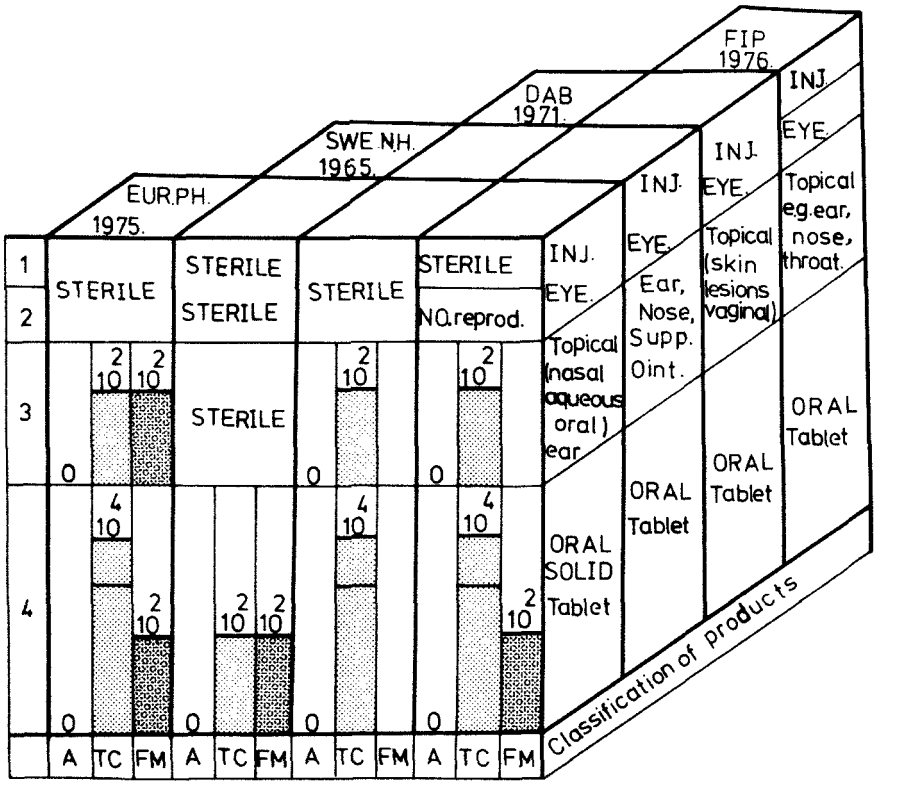
Microbiological Purity Standards for Pharmaceutical Products.



A=(E.coli, Staph. aureus, Ps.aeruginosa, Salmonella).

TC=Total Counts.

FM=Fungi & Mould.

EUR.PH. =EUROPEAN PHARMACOPOEIA.

SWEDISH NATIONAL HEALTH.

DIAGRAM.1

DAB=GERMAN PHARMACOPOEIA.

FIP = FÉDÉRATION INTER. PHARMACEUTIQUE.

salmonellosis to thyroid tablets, and later Fischer and others[2] reported counts of microorganisms as high as 10^5 per tablet in thyroid tablets. A number of studies have been reported, illustrating the wide range of materials used in tabletting which contain viable microorganisms.[3,4]

The drying processes during granulation and subsequent compression do in themselves contribute to a lowering of microbial content of tablets.[5,6] Further, the level of compression force by its effect upon pore size and consequent water relationships of the tablet will influence stability during storage.[7,8]

These factors together with precautions taken to prevent access of microorganisms during manufacture may be implemented by the incorporation of preservatives into tablets. In this paper, various methods of incorporating preservatives into tablets have been assessed by chromatographic estimation of final preservative content and by microbial challenge.

MATERIALS

Tablet ingredients:

Crystalline lactose, potato starch and magnesium stearate, (B.P. quality), methyl, ethyl and propyl p-hydroxybenzoic acid (parabens) and triphenyltetrazolium chloride (TTC), (British Drug Houses).

Tablet Machine:

A single punch machine (Manesty type F3) fitted with 12.5 mm flat punches was used to prepare tablets.

Chromatographic equipment:

A chromatographic tank was used with a solvent vessel placed 50 cm from the bottom. Whatmann No. 1 filter paper was used and an U.V. lamp, (Chromatolite, Gallenkamp), used for detection of pre-

servatives, photographic paper was obtained from Kodak (white smooth glossy 4S). The solvent systems used were, n-butanol, ammonia and water (5 : 2 : 3) and ethanol, ammonia, water (5 : 3 : 2). Cecil instrument CE272 - linear readout U.V. spectrophotometer was used for quantitative analysis of 'parabens'.

Organism and culture media:

The organism used was Aspergillus niger IMI 17454 (typical of spoilage forms) grown on malt extract agar (Oxoid CM59) at 25°. Spore suspensions were produced as described by Fassihi and Parker[7] and standardized to contain 8×10^6 spores/ml.

METHODS

(A) Preparation of tablets

Tablet ingredients were sterilized in an ethylene oxide chamber, (45°C, 65% R.H., 12 hours), Lactose tablets were prepared at $90MNm^{-2}$ compression force containing 5% w/w potato starch and 0.5% w/w magnesium stearate. In order to incorporate methyl, ethyl, propyl parabens or a mixture of methyl and propyl (2:1) parabens the following procedures were adopted:

a. Control tablets: Lactose, dried starch and magnesium stearate, mixed and compressed into tablets.

b. Dry mix incorporation: Lactose, mixed with approximately 0.06-0.1% w/w parabens, dried starch and magnesium stearate and compressed into tablets.

c. Wet mix incorporation: Lactose granulated with approximately 0.06-0.1% w/w parabens in sterile water, the wet mass discharged through 10 screen, dried at 50°C, screened through #16 mesh screen, starch and magnesium stearate added, mixed and compressed into tablets.

d. Spraying of granules with aqueous solution of parabens: Lactose was granulated with sterile water and the wet mass discharged through #10 screed dried at 50°C, screened through #16 mesh screen. The granules were rotated in a coating pan and approximately 0.06-0.1% w/v parabens in sterile water sprayed on, and after drying at 50°C for 2 hours, starch and magnesium stearate added, mixed and compressed into tablets.

e. Spraying of granules with alcoholic solution of parabens: The above procedure (d) was repeated using parabens dissolved in 95% ethanol to spray on granules.

In methods (c) and (d) it was necessary to heat the sterile water to incorporate the propyl parabens.

(B) Microbial challenge to tablets:

Challenge tests were performed with A. niger as the test organism. Five tablets from every batch were tested, by placing each tablet centrally on a malt extract agar plate and inoculating with 0.1 ml of A. niger spore suspension on the surface. The tablets were incubated at 25°C for 5 weeks and examined periodically for visible mould growth.

(C) Qualitative and quantitative analysis of preservatives in tablets:

1. Determination of R_f values of preservatives:

Standard solutions of methyl, ethyl and propyl parabens in ethanol were prepared and separation was made by descending paper chromatography, applying 150 µL of the standard solution of parabens, and allowing the chromatogram to develop for 5 to 8 hours at 25°C. The paper was air dried and superimposed upon a sheet of photographic

paper and exposed to U.V. light for a few seconds. On the developed print the position of the separated compounds appeared as white areas on a dark background. R_f values were determined (table 1).

2. Estimation of preservative level in tablets.

5 to 10 gram of crushed tablets was weighed into a 50 ml beaker and 20 ml ethanol was added and mixed thoroughly. Any undissolved particles were filtered off and filtrate was then evaporated to 2-10 ml on a steam bath under a jet of air. This concentrated solution was used for chromatography. The positions of the compounds on the chromatogram were determined by taking contact prints in U.V. light. The spots were eluted with ethanol and the absorbance of the eluates was measured spectrophotometrically and by reference to a standard graph the amount of parabens was estimated. (Table 2).

TABLE 1

*R_f values of parabens obtained from descending paper chromatography at 25°C

preservative	n-butanol, ammonia and water (5:2:3)		Ethanol, ammonia and water (5:3:2)		Detected by
	standard - sample		standard - sample		
Methyl paraben	0.78	0.79	0.64	0.64	UV
Ethyl paraben	0.84	0.84	0.66	0.67	UV
Propyl paraben	0.85	0.87	0.70	0.72	UV
Butyl paraben	0.87	0.86	0.74	0.74	UV

* $R_f = \frac{\text{Distance of spot from start point}}{\text{Distance of solvent front from start point}}$

Table (2). Microbial challenge resistance of tablets related to content of preservative and method of incorporation

Preservative	λmax	Approx.amount of preservative used %	Amount of preservative present % w/w/tablet Method of incorporation of preservative. b	c	d	e
Methyl paraben	255	0.10	0.10 (-)	0.09 (-)	0.080 (-)	0.09 (-)
		0.060	0.060 (+)	0.050 (+)	0.045 (+)	0.055 (+)
Ethyl paraben	258	0.10	0.10 (-)	0.075 (+)	0.090 (-)	0.075 (-)
		0.060	0.052 (+)	0.045 (+)	0.062 (+)	0.045 (+)
Propyl paraben	257	0.10	0.10 (-)	0.075 (-)	0.075 (-)	0.082 (-)
		0.060	0.062 (+)	0.045 (+)	0.055 (+)	0.042 (+)
Mixture of methyl		0.10	0.10 (-)	0.075 (-)	0.078 (-)	0.061 (±)
and propyl parabens (2:1)		0.060	0.065 (+)	0.042 (+)	0.056 (+)	0.060 (±)

(-) = no visible microbial growth, (+) = visible microbial growth. (±) = slight visible growth.
Tablets with no preservative supported microbial growth and broke down within three weeks.

Reduction of TTC as a measure of microbial contamination of tablets:

Tablets may be tested for indigenous microorganisms by sampling into broth and for survival of added bacteria by viable count techniques.[9] An alternative method used here for detecting microbial contamination is based upon the reduction of 2,3,5-Triphenyltetrazolium zoline chloride (TTC) to the red product formazan by actively growing microorganisms.

Sterile tablets and tablets which had been exposed to the atmosphere for two weeks were centrally placed on nutrient agar (oxoid No. 3) dishes and 0.1%w/v solution of TTC in sterile water was sprayed onto the surface of the tablets in a dark room. After incubation at 25°C for 8 hours a visible pink colour developed on the surface of those tablets which had previously been exposed to the air. Reflectance spectra were measured spectrophotometrically (S.P. 800 with attached Reflectance Accessory), (fig. 1).

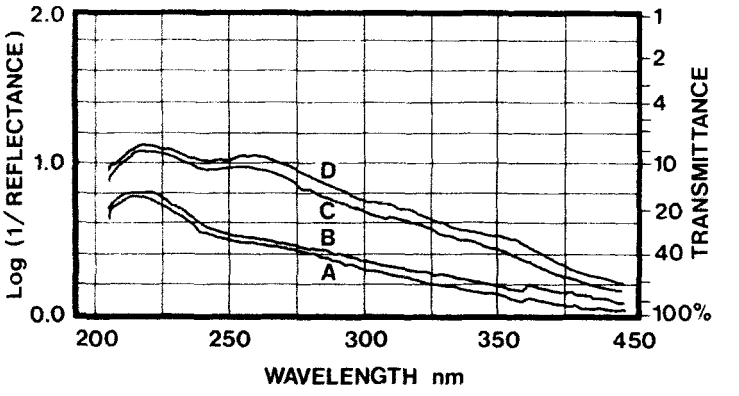


FIGURE 1

A – sterile tablet sprayed on with TTC
B – standard or sterile tablet
C&D – tablets exposed to atmosphere and sprayed on with TTC

The visible region gave an identical spectra for all tablets examined whether sterile or not, but differences in the U.V. region differentiated contaminated from non-contaminated tablets.

RESULTS AND DISCUSSION

Table 1, (The R_f values obtained for different solvent system) indicates that from the combination of R_f values, preservatives could be identified. The detection of the spots by photographic contact printing is easy and rapid and has been used by a number of investigators.[10,11]

The estimated amounts of preservatives in the tablets are summarized in Table (2). Recovery of preservatives was satisfactory although there was underestimation in some batches. This could be a result of interaction of parabens with starch or magnesium stearate, since adsorption and inactivation of a number of different antimicrobial agents, including parabens by these materials, has been reported.[12,13] Method (b), dry mixing of preservatives and granules gave the highest levels of preservative in the final tablets.

The incorporation of any of the parabens in the range of 0.075% to 0.1%$^{w}/_{w}$ in tablets is effective in protecting tablets against microbial spoilage, plates 1 to 6. The use of mixtures of the same concentration as the single esters offers no advantage. The method of assessing resistance to spoilage by visual examination is of necessity only a guide, although, as the plates indicate, clear end points are obtainable. Within the limit of this method our experience has been that spraying of the granules with either aqueous or alcoholic solution is the best method of incorporating preservative into tablets. This is interesting in that spraying methods (d) and (e) did not produce as high a level of parabens in the final tablets as the dry mixing technique (b). It seems that preservative availability is sufficiently enhanced when coated externally on to granules to recompense for losses inherent in this technique.

An alternative method to direct quantified microbial challenge of measuring resistance of tablets or of detecting microbial con-

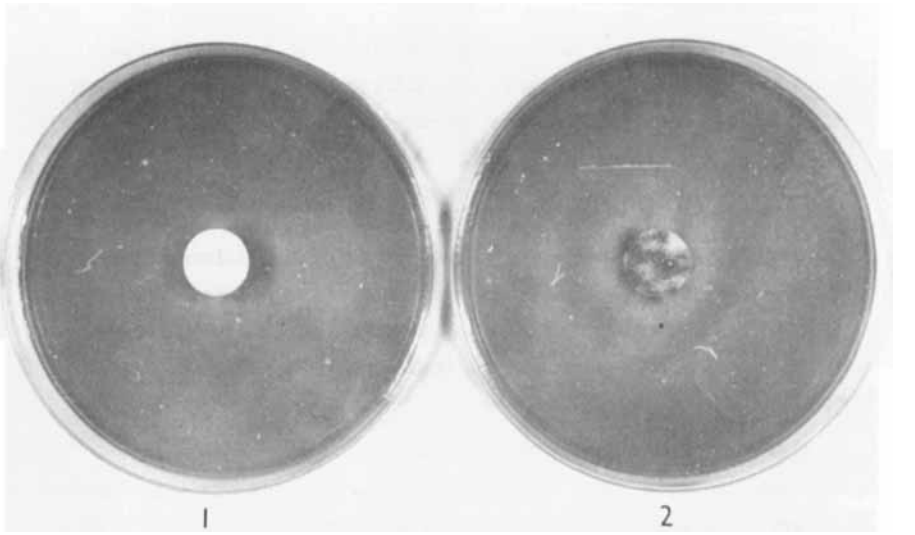


PLATE (1): 1. Tablet containing 0.1% w/w methyl paraben (Method b)
c. Control tablet without preservative

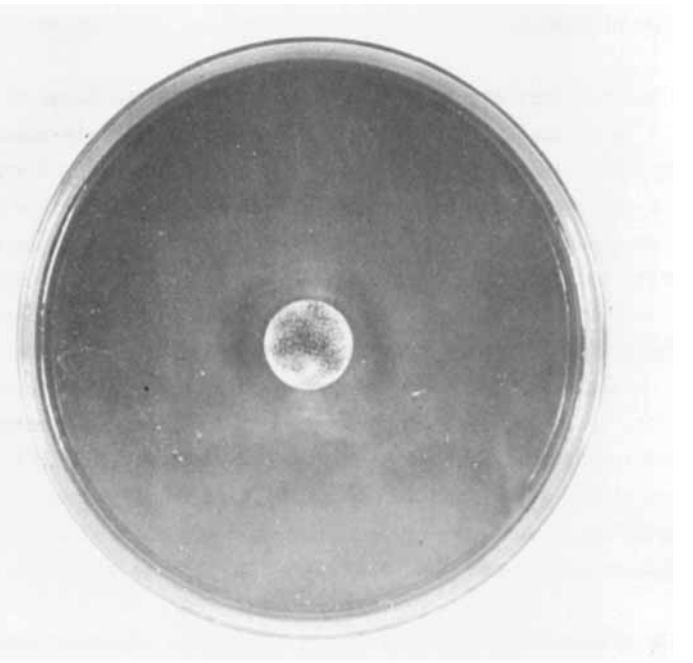


PLATE (2): Tablet containing 0.061% w/w methyl and propyl paraben (2:1) (Method e)

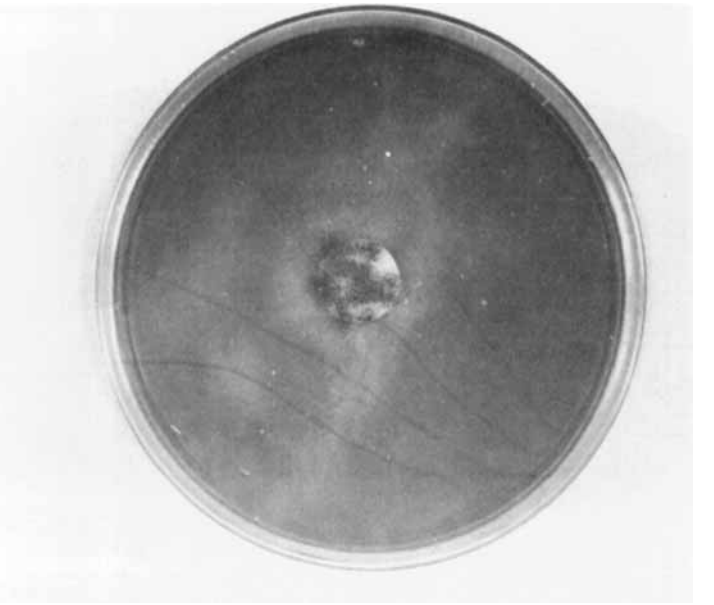


PLATE (3): Tablet containing 0.065% $^{w}/_{w}$ of methyl and propyl paraben (2:1) (Method b)

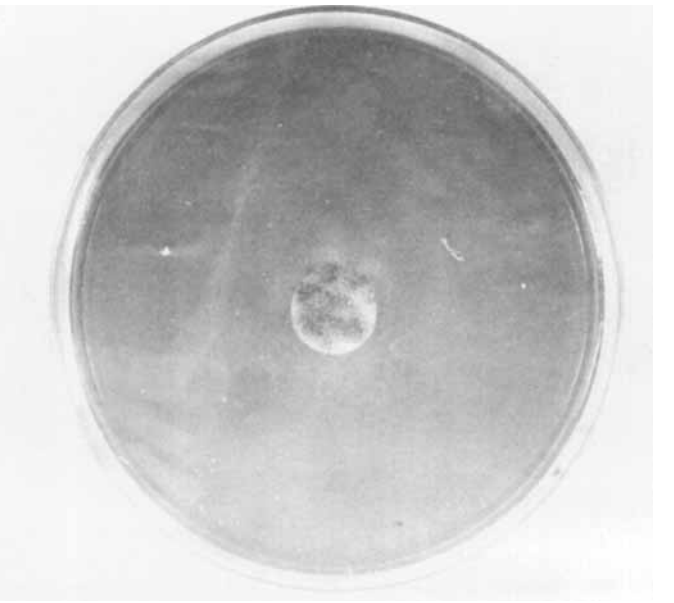


PLATE (4): Tablet containing 0.062% $^{w}/_{w}$ ethyl paraben (Method d)

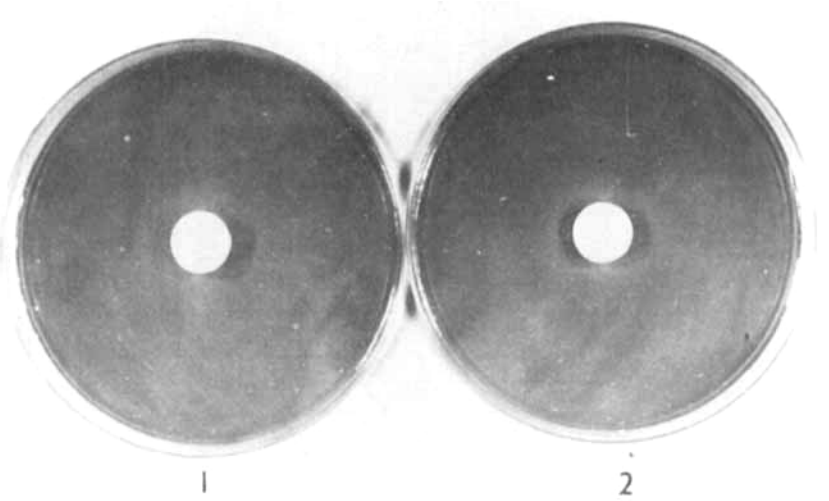


PLATE (5): 1. Tablet containing 0.080% $^{w}/_{w}$ methyl paraben (Method d)
2. Tablet containing 0.090% $^{w}/_{w}$ methyl paraben (Method c)

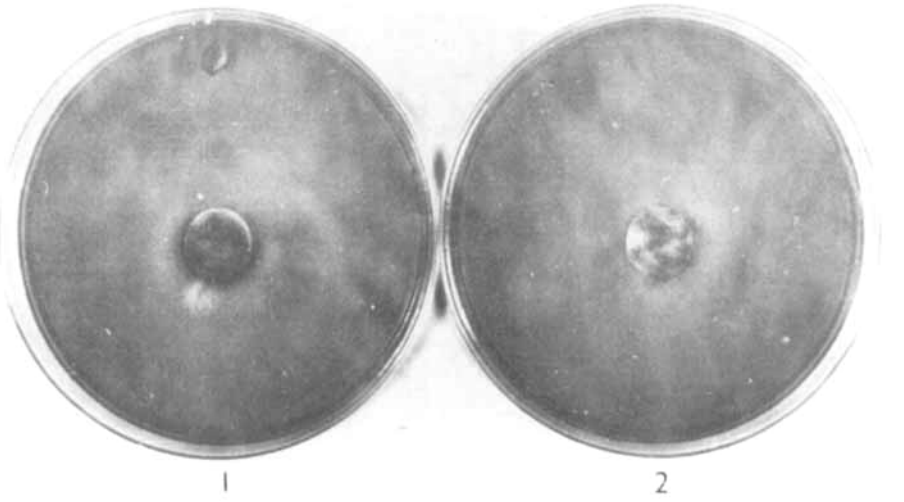


PLATE (6): 1. Tablet containing 0.075% $^{w}/_{w}$ ethyl paraben (Method c)
2. Tablet containing 0.055% $^{w}/_{w}$ propyl paraben (Method d)

tamination on them is to use TTC. The development of the pink formazan by viable organisms is well in advance of any appearance of visible growth. The method has been further refined here by measuring the reflectance of tablets after treatment with the reagent. Since a light sensitive reaction is involved reagent treatment and incubation should be carried out in the dark.

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