

**A PHARMACEUTICAL AND MICROBIOLOGICAL  
ANALYSIS OF MOUTHWASHES**

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## **Introduction**

Previous evaluation of mouthwashes, have very largely concentrated on determinations on the anti-plaque activity (Axelsson, 1987), their ability to control gingivitis, (Clark et al, 1987) and the effects of specific chemical agents used to reduce the bacterial count (Hogg, 1987). A pharmaceutical and microbiological analysis of 14 mouthwashes available in Malta were investigated, at St Luke's Hospital and the University of Malta.

## **Methodology**

Three studies were carried out over a 30 week period.

### **Study 1**

A pharmaceutical and stability analysis on Compound Thymol mouthwash, an extemporaneous product prepared at the Government Medical Stores was investigated in an attempt to solve the crystallization and precipitation that formed. This was assessed as follows:

1. Crystals were separated, purified and identified by physical and chemical methods.
2. The formulation of the product was slightly amended in order to improve the product from a pharmaceutical and chemical approach.
3. A stability study on the modified product was performed.

### **Study 2**

A. This study was developed on a method performed by Richards R. et al (1989) and was then compared with the results achieved in Malta. Furthermore this study was reported with additional data.

The reproducibility of a standardised rinse/gargle procedure (itemised below) was investigated by determining the aerobic bacterial count of 3 consecutive rinse/gargle routines following steps 1, 2 and 3 below using 5 subjects. Colony counts were performed on each of the samples by diluting appropriately in sterile water, spreading 0.1 ml on overdried blood agar

plates and incubating at 37°C for 48 hours. The standard deviation was calculated to check if the method could form the basis of an in vivo evaluation of mouthwashes on the bacterial flora of the mouth and throat. The standardised rinse/gargle procedure was as follows:

1. Take 15ml sterile deionised water in the mouth for a 30 second mouthwash by the following method:
    - (a) For 10 secs - gargle to back of throat;
    - (b) For 10 sec - using tongue, wash teeth, gums and cheeks mechanically;
    - (c) For 10 secs - rinse mouth.
  2. Expectorate the solution into a sterile container.
  3. Wait one minute.
  4.
    - (a) Repeat step 1 using 15ml of proprietary mouthwash provided;
    - (b) Expectorate the solution into the container provided containing 15ml of inactivator solution (3% Tween 80, 0.5% lecithin in thioglycollate broth USP);
    - (c) Wait one minute.
  5. Repeat steps 1 and 2 using the second 15ml volume of sterile deionised water.
  6. Return in 60 minutes (during which nothing should be consumed and repeat steps 1 and 2 using a third 15ml volume of sterile deionised water provided.
- B. The standardised rinse gargle procedure above was followed by 6 subjects randomly selected from the local hospital staff to evaluate each of 14 mouthwashes. Inactivated mouthwashes were plated to obtain colony counts as before.
- C. One mouthwash with poor and another with good antibacterial activity selected from the previous study were subjected to 3 physical parameters (time, temperature and volume) to investigate if the bacterial count was further reduced.

### Study 3

100 people were interviewed (62 females; 38 males) to investigate the public's knowledge and practices concerning mouthwashes. In addition

they were asked about the criteria of their choice of mouthwashes, who recommended the mouthwash and if they were aware of adverse effects that these products could possibly impart.

## Results

### Study 1

This pharmaceutical investigation showed that the hospital used a preparation that was several times amended by "The Extra Pharmacopoeia". In addition products in the formulation that had expired were still being used. Preparing the product with freshly compounded products still gave crystallisation on standing and on identification the crystals were found to be benzoic acid. This solubility problem was solved with the addition of more alcohol to the formulation and the reduction of water. With the new amendments made to the formulation the expiry date was 2 weeks which was rather short. Cooper and Gunn (1975) states that most extemporaneous solutions have a shelf life of approximately 1 month. The product could only be dispensed with the condition that it was to be discarded after 10 days. As the product used to be prepared in bulk, it would no longer be possible for the hospital to prepare it as it would be economically unfeasible.

### Study 2

The **reproducibility procedure** gave results which were satisfactory when compared with the U.K. in that they were fairly reproducible. The method employed indicates that it can form the basis of an "In-vivo" evaluation of the effects of mouthwashes on the aerobic bacterial flora of the mouth and throat. Figure 1 below are the comparative results.

The results of the mouthwash effectiveness is represented in Figure 2. This represents the pooled results for 6 subjects used to evaluate each of the 14 mouthwashes. The most effective mouthwashes had low numbers of viable organisms in the actual mouthwash rinse when compared with the initial number present in the pre-test evaluation. Furthermore, the sterile water used to rinse the mouth and throat one minute after using the effective mouthwash also contained comparatively low numbers of organisms. This represents a >99.5% reduction in colony forming units (C.F.U.) after one minute after the following mouthwashes were used: Oraldene<sup>R</sup>, Betadine<sup>R</sup>, Corsodyl<sup>R</sup>, Bocasan<sup>R</sup> and Listermint<sup>R</sup>. As for

Subject	United Kingdom		Malta	
	Average Count x 10 <sup>6</sup> ml	Standard Deviation	Average Count x 10 <sup>6</sup> ml	Standard Deviation
1	4.2	0.3	0.6	0.1
2	0.2	0.1	0.5	0.2
3	6.7	0.3	0.7	0.1
4	0.8	0.6	0.7	0.2
5	3.4	0.8	0.6	0.2

**Figure 1.** The results show the average count and the standard deviation (S.D.) of the aerobic bacterial flora of the mouth. The greater the variations of the (S.D.), the less valid of the test.

Listerine<sup>R</sup>, Dettol<sup>R</sup>, Acti-brush<sup>R</sup>, Reach<sup>R</sup>, Antoral<sup>R</sup>, Compound Thymol Glycerine, Saline, Hydrogen peroxide and Potassium permanganate the percentage reductions in C.F.U. were 83%, 30%, 96%, 92%, 86%, 37%, 84% and 41% respectively. After one hour the results for all products expressed as the percentage reductions in C.F.U. were Corsodyl<sup>R</sup> 93%, Oraldene<sup>R</sup> 92%, Betadine<sup>R</sup> 92%, Bocasan<sup>R</sup> 89%, Listerine<sup>R</sup> 31%, Listermint<sup>R</sup> 80%, Dettol<sup>R</sup> 3%, Acti-brush<sup>R</sup> 81%, Reach<sup>R</sup> 87%, Antoral<sup>R</sup> 75%, Compound Thymol Glycerine 72%, Saline 11%, Hydrogen peroxide 29% and Potassium permanganate 26%.

From the above mouthwashes, only six were similarly studied in the U.K. as the others are either non-proprietary mouthwashes or are recent products that were on the market since two years ago. The comparative results below, show that they are closely correlated (Corsodyl<sup>R</sup>: Malta 93%; U.K. 89%); (Oraldene<sup>R</sup>: Malta 92%; U.K. 91%); (Betadine<sup>R</sup>: Malta 92%; U.K. 91%); (Bocasan<sup>R</sup>: Malta 89%; U.K. 88%); (Listerine<sup>R</sup>: Malta 29%; U.K. 22%); (Dettol<sup>R</sup>: Malta 3%; U.K. 0%).

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Mouthwash Percentage Effectiveness

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Mouthwash	After 1 min.	After 1 hr
- Corsodyl	99.5	93.0
- Oraldene	99.5	92.0
- Betadene	99.5	92.0
- Bocasan	99.5	89.0
- Listerine	83.0	31.0
- Listermint	99.5	80.0
- Dettol	30.0	3.0
- Acti-brush	96.0	81.0
- Reach	92.0	87.0
- Antoral	86.0	75.0
- Saline	37.0	11.0
- Hydrogen peroxide	84.0	29.0
- Permanganate	41.0	26.0
- Compound Thymol Glycerine	86.0	72.0

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**Figure 2.** The results show the percentage effectiveness of the 14 mouthwashes analysed. Each mouthwash was analysed by 6 subjects and the average percentage count was calculated.

Listermint and Listerine were the selected mouthwashes from the previous study. These were exposed to the following physical proportions: The volume, time and temperature from 15mls, 30 secs and 25°C were increased to 50mls, 4 mins and 45°C. The following observations were made: **For Volume** there was a 5.7%, **for temperature** there was a 10.4% and **for time** there was an 8% further reduction in the bacterial count from the original 80% for Listermint<sup>R</sup>. For Listerine<sup>R</sup> it was 5.8%, 7/78% and 3.44% respectively from the original 30% of the bacterial count.

### Study 3

100% of the patients (n=100) responded to the questionnaire. 30% used a mouthwash of which 20% (n=30) use a particular brand. 20% (n=100)

had a mouthwash prescribed by a dentist and pharmacists or doctors do not prescribe such products. 50% (n=100) knew the difference between a gargle and a mouthwash while 90% (n=100) knew that such products contained some form of medication. Only 30% (n=100) knew that such products imparted side-effects if over used. 10% (n=100) bought mouthwashes to treat halitosis (bad breath) or to treat stomatitis, 80% (n=100) to treat ulcers or dental caries while 40% use them to keep their mouth clean. 70% (n=100) consider price, flouride content and manufacturer's claims as the criteria for their choice of buying a mouthwash while 40% (n=100) consider taste as well. All people questioned (n=100) agreed that more health education is required concerning these products.

### **Discussion and Conclusion**

To achieve the acquired standards of oral health it is necessary for a pharmacist as an educator to know what a truly effective mouthwash is. Both pharmaceutical and antibacterial properties, should be taken into consideration.

The pharmaceutical analysis showed that mouthwashes must be correctly prepared, stored and periodically tested by some forms of quality control. Such procedures ensure that it reaches the patient safely and offers its full activity when used. Suggestions to substitute Compound Thymol mouthwash to Compound Thymol Glycerine was recommended as this product has a recognised B.P. standard assay so that it could be routinely analysed.

The microbiological analysis gave an idea about the antibacterial effectiveness and what physical conditions exposed to these mouthwashes would further their activity.

The survey indicates that the public requires oral health education concerning mouthwashes. In addition dispensing the correct mouthwash and giving the necessary advice for use in combination with toothbrushing procedures will help to promote high standards of oral health. The pharmacist is the likely candidate to bring this into action.

## References

Axelsson P., Lindhe J. Efficacy of mouthrinses in inhibiting dental plaque and gingivitis in man. *The Journal of Clinical Periodontology* 1987; 14: 205-212.

Clark W., Magnusson I., Walker C., McArthur W., Marks R., Wheeler T. Efficacy of Perimed antibacterial system on established gingivitis. *The Journal of Dental Research* 1987; 66: 153 (abstract 372).

Hogg D.S. Chemical control of plaque. *Dental Update* 1990; 8: 330-334.

Richards R.M.E., Cowie G., McCague. In vivo investigations of the antibacterial activity of lozenges and mouthwashes on the aerobic bacterial flora of the mouth and throat. *The Pharmaceutical Journal* 1989; 6: 669-663.

Cooper and Gunn (1975). *Dispensing for pharmaceutical students*. Singapore, Longman. 9: 85.